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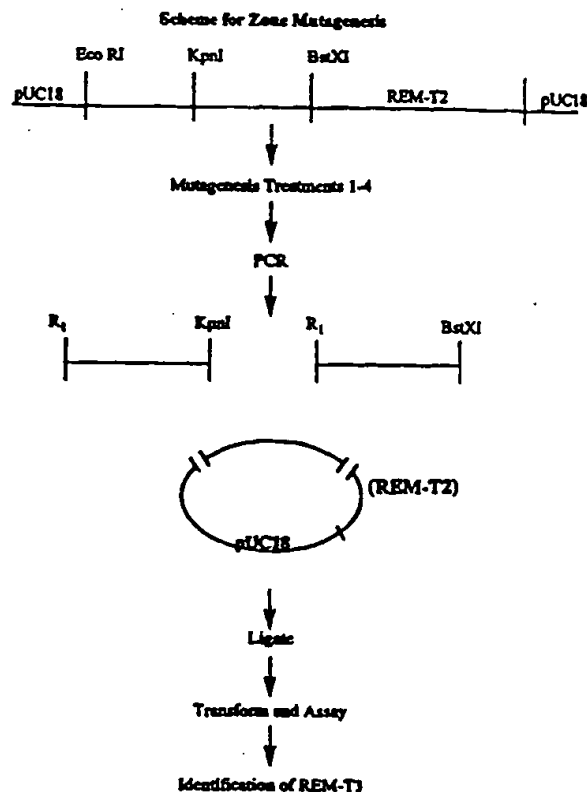
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(71) Applicant (for all designated States except US): STATE OF OREGON acting by and through THE OREGON STATE BOARD OF HIGHER EDUCATION on behalf of THE OREGON HEALTH SCIENCES UNIVERSITY [US/US]; 3181 S.W. Sam Jackson Park Road, Portland, OR 97201-3098 (US).			
(72) Inventor; and (75) Inventor/Applicant (for US only): MOSES, Robb, Edwin [US/US]; 2771 S.W. Patton Lane, Portland, OR 97201 (US).			
(74) Agent: GREENFIELD, Michael, S.; Allegetti & Witcoff, Ltd., Ten South Wacker Drive, Chicago, IL 60606 (US).			

(54) Title: A MODIFIED THERMO RESISTANT DNA POLYMERASE

(57) Abstract

Novel, modified *Taq* DNA polymerases and genes encoding for them are disclosed. The modified *Taq* DNA polymerases of the invention are the same size, have the same heat stability and synthesis rate as the native enzyme, but lack the 5'-3' exonuclease activity. As a result of this modification, the enzymes have improved processivity as compared to the native enzyme. The enzymes of the present invention enable improved methods of conducting PCR, DNA sequencing, and DNA synthesis.



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MODIFIED THERMO-RESISTANT DNA POLYMERASES

BACKGROUND OF THE INVENTION

"This invention was made with Government support under grant GM 24711 awarded by the National Institutes of Health. The Government has certain rights in the invention."

5 Field of the Invention

This invention relates to the field of DNA polymerases for use in the polymerase chain reaction and DNA sequencing.

Description of the Prior Art

10 Polymerase Chain Reaction (PCR) was one of the most important inventions developed in area of biotechnology during the 1980's and has proven useful for a variety of tasks. *PCR Technology, Principles and Applications for DNA Amplification* (Erlich ed. 1989). The process provides a method for amplifying known specific nucleic acid sequence. Mullis, U.S. Pat. No. 4,683,202. The process comprises treating single or double stranded DNA containing the sequence of interest with an excess of two
15 oligonucleotide primers sufficiently complementary of the strands so as to hybridize to the denatured strands. The hybridized primers are then extended by a DNA polymerase in the presence of the four dNTPs. The primer extension products are then separated and can serve as templates for another cycle of replication. The number of DNA templates approximately doubles on each cycle of amplification. Thus, 20 cycles of the
20 process will result in approximately a 2^{20} -fold amplification.

The original protocols for PCR used the Klenow fragment of *E. coli* DNA polymerase I to catalyze the extension of the oligonucleotide primers. Mullis et al., *Cold Spring Harbor Symp. Quant. Biol.* 51, 263 (1986); Mullis and Faloona, *Methods*

Enzymol. 155, 335 (1987). The Klenow fragment proved somewhat cumbersome to use. Denaturation of the double stranded DNA at the start of each cycle requires temperatures ranging from 80 to 105°C. These temperatures inactivate the Klenow fragment. Consequently, fresh enzyme was required at the start of each new amplification cycle. While this process generally worked well for small segments of DNA (< 200 bp), a host of problems arose when replication of larger fragments was attempted.

The difficulties associated with use of the Klenow fragment DNA polymerase were circumvented with the introduction of thermostable DNA polymerase obtained from the thermophilic bacterium *Thermus aquaticus* (*Taq* DNA polymerase). Saiki et al., *Science* 239, 487 (1989); Gelfand et al., U.S. Pat. No. 4,889,818. This enzyme has been cloned, overproduced, and the DNA sequence determined. Lawyer et al., *J. Biol. Chem.* 264, 6427-6437 (1989).

In addition to its DNA polymerase activity, *Taq* DNA polymerase also possesses 5'-3' polymerization-dependent exonuclease activity, but it lacks 3'-5' exonuclease activity. Longley et al., *Nuc. Acids Res.* 18, 7317-7322 (1990); Blanco et al., *Gene* 100, 27-38 (1991); Bernad et al., *Cell* 59, 219-228 (1989); Lawyer et al., *supra*; Holland et al., *Proc. Natl Acad. Sci.* 88, 7276-7280 (1991); and Kelly and Joyce, *J. Mol. Biol.* 164, 529-560 (1983). Studies have identified the 5'-3' exonuclease activity as being an intrinsic part of *Taq* DNA polymerase. Longely et al., *supra*; and Barnes et al., *Gene* 112, 29-35 (1992). This activity appears to facilitate a nick translation DNA reaction.

Native *Taq* DNA polymerase suffers from a high rate of misincorporation — about four times higher than that of the Klenow fragment of *E. coli* DNA polymerase I.

It has been estimated that *Taq* DNA polymerase incorporates one incorrect nucleotide in 9000. Tindall and Kunkel, *Biochemistry* 27, 6008 (1988). After 20 amplification cycles, this would result in DNA molecules with random mutations averaging one in every 900 bases. Saiki et al., *supra*. If the PCR product is to be inserted into an expression vector, the chance that one cloned molecule will contain an unwanted sequence alteration may be significant. It would be desirable, therefore, to decrease the rate of misincorporation of the DNA polymerase used in PCR without sacrificing the heat stability and rate of synthesis of the native *Taq* DNA polymerase.

It has been shown that removal of the 5'-235 codons of the *Taq* DNA polymerase gene results in an expression product that has no 5'-3' exonuclease activity and a lower rate of mutagenesis. Tindall et al., *supra*; and Barnes, *supra*.

Other forms of *Taq* DNA polymerase are available. AmpliTaq™ is a commercially available genetically engineered version of *Taq* DNA polymerase and is substantially equivalent to the native form. Perkin Elmer Cetus; Saiki and Gelfand, *Amplifications* (Perkin Elmer Cetus), 1, 4 (1989). Also commercially available is a truncated gene product, the Stoffel fragment, that expresses an enzyme lacking the 5'-3' exonuclease activity and having much lower unit activity, probably due to decreased processivity and increased mutagenesis. Barnes, *supra*. Gelfand and Abramson (PCT International Publication No. WO 92/06200) disclosed a modified *Taq* polymerase having the same length as the native enzyme, but with highly attenuated 5'-3' exonuclease activity. The exonuclease activity is defeated by mutation in nucleotide 137 of the *Taq* polymerase gene, wherein the mutation is G

to A, resulting in a change in amino acid 46 of the enzyme from Gly to Asp. This enzyme is reported as having the same polymerase activity, processivity and extension rate as the native enzyme.

SUMMARY OF THE INVENTION

5 An object of this invention is to enhance the synthesis activity of DNA polymerase as used in PCR and DNA sequencing.

 The invention disclosed herein achieves this object by providing a modified *Taq* DNA polymerase and a correspondingly modified *Taq* DNA polymerase gene sequence. The modified *Taq* DNA polymerase is the same size, has the same heat
10 stability and synthesis rate as the native enzyme, but the 5'-3' exonuclease activity is missing. As a result of this modification, the gene expression product has improved processivity.

 The enzymes of the present invention enable improved methods of conducting PCR, DNA sequencing and DNA synthesis.

15

BRIEF DESCRIPTION OF THE DRAWINGS

 Figure 1 is a graphical depiction of the restriction map of the *Taq* DNA polymerase gene.

 Figure 2 is a graphical depiction of the method for producing the modified *Taq* DNA polymerase and the gene encoding it.

20

 Figure 3 shows the sequencing primers for the pLSM5 (SEQ ID NO: 3) plasmid.

Figure 4 is a schematic depiction of the method for testing processivity used in trials 1 and 2.

Figure 5 is the autoradiograph showing the results of processivity testing used in trial 1.

5 Figure 6 is the autoradiograph showing the results of processivity testing used in trial 2.

Figure 7 is a schematic depiction of the method for testing processivity using PCR.

10 Figure 8 is the autoradiograph showing the results of processivity testing by the PCR method.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the term "replication product" refers to the oligonucleotides synthesized by DNA polymerase, whether it be as part of the polymerase chain reaction, DNA sequencing, or any other reaction where DNA polymerase is used to
15 synthesize an oligonucleotide.

The term "oligonucleotide" as used herein is defined as a molecule composed of two or more deoxyribonucleotides or ribonucleotides.

The term "thermostable" refers to an enzyme that is stable to heat ($> 95^{\circ}\text{C}$) and catalyzes combination of nucleotides to form an oligonucleotide. The term
20 "thermo stability" as used herein refers to the characteristic stability of an enzyme to heat.

As used herein, the term "altered amino acid" means an amino acid that differs from that found in the native peptide or protein. Hence, if the native peptide

has the amino acid Cys at position 43, and the modified peptide has the amino acid Gly at that position, Gly is the "altered amino acid." Similarly, the term "altered nucleotide" means a nucleotide that differs from that found in a native oligonucleotide, polynucleotide, gene, or other nucleotide fragment.

5 As used herein, the phrase "lacking 5'-3' exonuclease activity" means an enzyme having less than 1% of the 5'-3' exonuclease activity of the native *Taq* DNA polymerase.

We undertook to inactivate the 5'-3' exonuclease activity of the *Taq* DNA polymerase by *in vitro* mutagenesis without removal of the portion of the gene encoding that activity. The procedure followed was to develop a method of "zone
10 mutagenesis" for that region of the *Taq* DNA polymerase gene encoding for the 5'-3' exonuclease activity. See Figure 2. The nucleic acids encoding the amino acid residues required for 5'-3' exonuclease activity have not been clearly identified, but earlier work suggests the region involved in DNA polymerases from other bacteria.
15 Kelly and Joyce, *supra*.

To briefly summarize, using PCR technology we generated a *Taq* gene, which we cloned into the plasmid vector pUC18. See Figure 1. The pUC18 plasmid containing the *Taq* gene is designated pLSM5 (SEQ ID NO: 3). There are four base changes in the *Taq* gene produced by PCR and cloned in pLSM5 (SEQ ID NO: 3)
20 compared to the published *Taq* DNA polymerase gene sequence (TTHTAQPIA in GenBank) (SEQ ID NO: 1): 1) C to G at position 89 in the untranslated 5' end, 2) T to A at position 934 (Phe to Ile), 3) T to C at position 962 (Leu to Pro), and 4) G to A (no amino acid change) at position 2536. The protein expression product of this

gene has an altered amino acid at positions 272 (Ile) and 281 (Pro). We then subjected the pLSM5 (SEQ ID NO: 3) plasmid to conditions that would cause the random mutations in the 5' exonuclease domain.

5 The vector encoding the *Taq* gene (pLSM5 (SEQ ID NO: 3) producing the enzyme REM-T2 (SEQ ID NO: 4)) begins at nucleotide 70 and ends at 2619. The reading frame for translation begins at nucleotide 121 and ends at 2619 by the convention of Lawyer et al., *J. Biol. Chem.* 264, 6427-6437 (1989).

The following sequence appears at the 5' junction between the pUC18 plasmid and the *Taq* gene:

10 ... AATTTCACACAGGAAACAGCTATGACCATGATTACGAAATTCTAAA ...
(SEQ ID NO: 14)

This sequence begins with the pUC18 antisense nucleotide sequence 490 to 455. The underlined nucleotides (AA) were added to create a restriction site. The *Taq* gene sequence (bold face) begins at nucleotide 70).

15 The following sequence appears at the 3' junction between the pUC18 plasmid and the *Taq* gene:

...CAAGGAGTGAGGATTCTCTAGAGTCGACCTGCAGGCATGCAA
GCTTGGCACTGGCCGTCGTTTT ... (SEQ ID NO: 15)

20 This sequence begins with *Taq* polymerase gene nucleotide 2610 to 2619. The underlined nucleotides (GA) were added to create a restriction site. The remaining sequence is the pUC18 antisense nucleotide, 413 to 381. Both junction sequences have been verified by sequence analysis.

The enzyme expression product of the pLSM5 plasmid, REM-T2 (SEQ ID
25 NO: 4), has substantially the same processivity, 5'-3' exonuclease activity, and

performance in normal PCR, to the extent tested so far, as the commercially available *Taq* DNA polymerase AmpliTaq™.

A variety of methods of mutagenesis are known to those of skill in the art and may be used in preparing a modified *Taq* DNA polymerase gene according to the present invention. See, e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, 2d Ed. 1989). The mutated genes were then treated with restriction endonucleases that cut it in the region believed to be responsible for 5'-3' exonuclease activity, thereby producing mutated inserts coding for that portion of the gene. A vector containing the native *Taq* DNA polymerase gene was treated with the same endonucleases and the previously obtained inserts ligated into the vector. Cells were transformed with the vector containing the inserts and colonies grown. We assayed polymerases expressed by the various colonies for polymerase activity as well as 5'-3' exonuclease activity. The cells transfected with the gene encoding the modified *Taq* DNA polymerase meeting the objective of the present invention were thereby identified.

Appropriate host cells for the present invention may be chosen from the prokaryote group, which most frequently are represented by various strains of *E. coli*. Other microbial strains such as bacilli may be used, however. *Bacillus subtilis* and various species of *Pseudomonas* may be used, for example.- In such prokaryotic systems, plasmid vectors that contain replication sites and control sequences derived from a species compatible with the host are used. For example, *E. coli* is typically transformed using derivatives of pBR322, a plasmid derived from an *E. coli* species by Bolivar, et al., *Gene* 2, 95 (1977). pBR322 contains genes for ampicillin and tetra-

cycline resistance, and thus provides addition markers that can be either retained or destroyed in constructing the desired vector. Commonly used prokaryotic control sequences, which are defined herein to include promoters for transcription initiation, optionally with an operator, along with ribosome binding site sequence, include such commonly used promoters as the β -lactamase (penicillinase) and lactose (lac) promoter systems (Chang, et al., *Nature* 198, 1056 (1977)), the tryptophan (trp) promoter system (Goeddel, et al., *Nucleic Acids Res.* 8, 4057 (1980)), the lambda-derived P_L promoter (Shimatake et al., *Nature* 292, 129 (1981)), and the N-gene ribosome binding site, which has been made useful as a portable control cassette (U.S. Pat. No. 4,711,845). The N-gene ribosome binding site comprises a first DNA sequence that is the P_L promoter operably linked to a second DNA sequence corresponding to N_{RBS} upstream of a third DNA sequence having at least one restriction site that permits cleavage within six bp 3' of the N_{RBS} sequence. Also useful is the phosphatase A (phoA) system described by Chang et al. in European Patent Publication No. 196,864 published Oct. 8, 1986. Any available promoter system compatible with prokaryotes can be used, however.

In addition to bacteria, eucaryotic microbes, such as yeast, may also be used as hosts. Laboratory strains of *Saccharomyces cerevisiae*, Baker's yeast, are most used, although a number of other strains are commonly available. While vectors employing the 2 micron origin of replication are illustrated (Brach, *Meth. Enz.* 101, 307 (1983)), other plasmid vectors suitable for yeast expression are known (see, e.g., Stinchcomb et al., *Nature* 282, 39 (1979), Tschempe et al., *Gene* 10, 157 (1980), and Clarke et al., *Meth. Enz.* 101, 300 (1983). Control sequences for yeast vectors include promoters

for the synthesis of glycolytic enzymes. Hess et al., *J. Adv. Enzyme Reg.* 7, 149 (1968) and Holland et al., *Biotechnology* 17, 4900 (1978).

Additional promoters known in the art include the promoter for 3-phosphoglycerate kinase (Hitzeman et al., *J. Biol. Chem.* 255, 2073 (1980) and those for other glycolytic enzymes, such as glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. Other promoters that have the additional advantage of transcription controlled by growth conditions are the promoter regions for alcohol dehydrogenase 2, isocytichrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, and enzymes responsible for maltose and galactose utilization. Holland, *supra*.

It is also believed that terminator sequences are desirable at the 3' end of the coding sequences. Such terminators are found in the 3' untranslated region following the coding sequences in yeast-derived genes. Many of the vectors illustrated contain control sequences derived from the enolase gene containing plasmid peno46 (Holland et al., *J. Biol. Chem.* 256, 1385 (1981) or the LEU2 gene obtained from YEp13 (Broach et al., *Gene* 8, 121 (1978). Any vector containing a yeast-compatible promoter, origin of replication, and other control sequence is suitable, however.

It is also possible to express genes encoding polypeptides in eucaryotic host cell cultures derived from multicellular organisms. See, e.g., *Tissue Culture* (Cruz and Patterson eds., Academic Press 1973). Useful host cell lines include murine myelomas N51, VERO and HeLa cells, and Chinese Hamster Ovary (CHO) cells.

Expression vectors for such cells ordinarily include promoters and control sequences compatible with mammalian cells such as the commonly used early and late promoters from Simian Virus 40 (SV 40) (Fiers et al., *Nature* 273, 113 (1978)) or other viral promoters such as those derived from polyoma, Adenovirus 2, bovine papilloma virus, or avian sarcoma viruses, or immunoglobulin promoters and heat shock promoters. A system for expressing DNA in mammalian systems using the BPV as a vector is disclosed in U.S. Pat. No. 4,419,446. A modification of this system is described in U.S. Pat. No. 4,601,978. General aspects of mammalian cell host system transformations have been described by Axel, U.S. Pat. No. 4,399,216. It now appears that "enhancer" regions are important in optimizing expression. These generally are sequences found upstream of the promoter region. Origins of replication may be obtained from viral sources. Integration into the chromosome, however, is a common mechanism for DNA replication in eucaryotes.

Plant cells are also now available as hosts. Control sequences compatible with plant cells such as the nopaline synthase promoter and polyadenylation signal sequence are available. Depicker et al., *J. Mol. Appl. Gen.* 1, 561 (1982).

In addition, expression systems employing insect cells utilizing the control systems provided by baculovirus vectors have been described. Miller et al, *Genetic Engineering* 8, 277-297 (Setlow et al. eds. Plenum Publishing 1986). These systems are also successful in producing *Taq* DNA polymerase.

Cells transformed with the modified *Taq* DNA polymerase gene may be grown using any suitable technique. The appropriate technique will depend on the cell type and will be known to those skilled in the art.

Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The treatment employing calcium chloride is used for prokaryotes or other cells that contain substantial cell wall barriers. Cohen, *Proc. Natl. Acad. Sci. (USA)* 69, 2110 (1972). Infection with *Agrobacterium tumefaciens* is used for certain plant cells. Shaw et al. *Gene* 23, 315 (1983). For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb is preferred. *Virology* 52, 546 (1978). Transformations into yeast are carried out according to the method of Van solingen et al., *J. Bact.* 130, 946 (1977) and Hsiao et al., *Proc. Natl. Acad. Sci. (USA)* 76, 3829 (1979).

Construction of suitable vectors containing the desired coding and control sequences employs standard ligation and restriction techniques that are well understood in the art. Isolated plasmids, DNA sequences, or synthesized oligonucleotides are cleaved, tailored, and relegated in the form desired.

Site-specific DNA cleavage is performed by treating with the suitable restriction enzyme (or enzymes) under conditions that are generally understood in the art, and the particulars of which are specified by the manufacturer of these commercially available restriction enzymes. See, e.g., *New England Biolabs, Product Catalog*. In general, about 1 μ g of plasmid or DNA sequence is cleaved by one unit of enzyme in about 20 μ l of buffer solution. Often excess of restriction enzyme is used to ensure complete digestion of the DNA substrate. Incubation times of about one hour to two hours at about 37°C are workable, although variations are tolerable. After each incubation, protein is removed by extraction with phenol/chloroform, and may be followed by ether extraction. The nucleic acid may be recovered from

aqueous fractions by precipitation with ethanol. If desired, size separation of the cleaved fragments may be performed by polyacrylamide gel or agarose gel electrophoresis using standard techniques. A general description of size separations is found in *Methods in Enzymology* 65, 499-560 (1980).

5 Cells producing enzyme of the desired type can be identified by standard techniques for assaying DNA polymerase and 5'-3' exonuclease activity. *Id.* Using some of these methods, we were able to isolate a *Taq* DNA polymerase having the same size, heat stability, and synthetic activity of native *Taq* DNA polymerase, but having increased processivity and resulting in decreased mutagenesis of PCR DNA
10 products. *See examples infra.*

 The modified *Taq* DNA polymerase of the present invention was chosen from a colony producing the enzyme with a relatively high polymerase activity and low 5'-3' exonuclease activity. We designated this product REM-T3 (SEQ ID NO: 6). An equivalent independently isolated product with a different mutation but equivalent
15 properties is designated REM-T5 (SEQ ID NO: 8).

 In addition to the modifications of native *Taq* DNA polymerase present in the modified *Taq* DNA polymerase of the present invention, individual amino acid residues in the peptide chain comprising the *Taq* DNA polymerase may be modified or deleted without eliminating any of the requisite properties described herein. Such
20 alterations that do not destroy activity do not remove the DNA sequence or the modified *Taq* DNA polymerase from the contemplated scope of the present invention.

In order to assay the modified *Taq* DNA polymerase, REM-T3 (SEQ ID NO: 6), it was necessary to isolate it. We used the following novel, short isolation technique producing high purity enzyme quickly. Bacteria were grown overnight or to an OD at 600 nm of about 2.0 to 2.5 and then centrifuged at 5000 rpm for 10 minutes. The supernatant was discarded and the pellet washed with a solution of 50 mM Tris(8.0), 50 M dextrose, and 1 mM EDTA (15 x cell wt). The pellet was re suspended and lysed with a solution of 50 mM Tris, 50 mM dextrose, 1 mM EDTA, and 1 mg/ml lysozyme(5x cell wt). An equal volume of a solution of 10 mM Tris and 50 mM KCl, and 1 mM EDTA was added and the resulting mixture incubated at 75°C for 60 min before centrifuging at 8000 rpm for 15 min. The pellet was discarded and an equal volume of DEAE and 0.4 M KPO₄ (6.8) was added to the supernatant. The mixture was then incubated at 0°C for 30 min and then centrifuged at 10,000 rpm for 20 min. The pellet was discarded and the supernatant put on a phosphocellulose column with 0.02 M KPO₄ (7.5)(4x cell wt). The column was eluted with a gradient of 0.02 to 0.4 M KPO₄ (7.5). The peak was collected and applied to a Bio Rex-70 column with a solution of 0.02 M KPO₄ (7.6), 80 mM KCl 5%, glycerol, 0.5% Tween, and 0.5% Nonidet P-40. This column was then eluted with a step gradient of 0.3 M KCl and the peak collected.

The thermostability of the modified *Taq* DNA polymerase of the present invention must be substantially equivalent to that of native *Taq* DNA polymerase, i.e., it must not become irreversibly denatured (inactivated) when subjected to the elevated temperatures for the time necessary to effect denaturation of double-stranded nucleic acids. The heating conditions (e.g., temperature and time) necessary

for denaturation will depend on a variety of factors, including the buffer salt concentration and the length and composition of the nucleotide chain. Typically, the temperature range for which the enzyme must be stable is about 90 to about 105°C for about 0.5 to four minutes. These values may vary depending on the conditions.

5 The modified *Taq* DNA polymerase of the present invention preferably functions optimally at temperatures above 40°C. The enzymes of the present invention is active in the temperature range 55 - 95°C, and preferably in the range 70 - 95°C.

10 U.S. Pat. No. 4,889,818 discloses and claims a native form of *Taq* DNA polymerase. Because the modified *Taq* DNA polymerase of the present invention retains all the characteristics of the native form that are useful in PCR technology, its use in PCR is preferable to the native form. Consequently, applications using *Taq* DNA polymerase as described in U.S. Pat. No. 4,889,818, col. 14, l. 33 to col. 27, l. 27 may also use the modified *Taq* DNA polymerase of the present invention.

15 Accordingly, the disclosure of U.S. Pat. No. 4,889,818 is hereby incorporated by reference.

Besides use in the polymerase chain reaction, the modified *Taq* DNA polymerase of the present invention can be used in DNA sequencing by, for example, the Sanger dideoxy-mediated chain-termination method. Sanger et al., *Proc. Natl. Acad. Sci.* 74, 5463 (1977). Other similar uses will be known to those of skill in the art.

20

The following examples further elucidate the present invention, but are not intended to limit it.

EXAMPLE 1

Zone Mutagenesis of the *Taq* DNA Polymerase Gene - Treatment 1

The *Taq* polymerase gene was amplified from genomic DNA (*Thermus aquaticus*) using primers adding an EcoRI site in the 5' UTR (nucleotide 70) and BglII site at the 3' end (nucleotide 2619). The the PCR product was cloned into pUC18 after digesting the vector with EcoRI and BamHI. See Figure 1. We designated this *Taq* gene REM-T2. We then incubated the plasmid containing the *Taq* gene at pH 4.8 (10 mM sodium acetate) and room temperature for 20 minutes followed by neutralization to pH 8.0 with 50 mM Tris HCl. Inserts for the putative amino terminal region of the gene were generated by PCR using the "reverse primer" for pUC18 (CAG GAA ACA GCT ATG ACC (SEQ ID NO: 11) and the "sequencing primer" 628A (CCC AAA GCC AGG CCG (SEQ ID NO: 12)) followed by digestion with Eco RI and KpnI.

The pLSM5 (SEQ ID NO: 3) vector was digested with EcoRI and KpnI and purified. The inserts previously generated were then inserted into this vector. Ligation for insertion of the modified *Taq* gene was followed by transformation of DH5a cells followed by growth of individual colonies with assay for the DNA polymerase activity and the 5'-3' exonuclease activity.

EXAMPLE 2

Zone Mutagenesis of the *Taq* DNA Polymerase Gene - Treatment 2

Using PCR, we generated a *Taq* gene (REM-T2), which we cloned into the plasmid vector pUC18. See Figure 1. We incubated the plasmid DNA containing the

Taq gene at pH 4.8 and 60°C for 5 minutes followed by neutralization to pH 8.0 with 50 mM Tris HCl. Inserts for the putative amino terminal region of the gene were generated by PCR using the "reverse primer" for pUC18 (CAG GAA ACA GCT ATG ACC (SEQ ID NO: 11)) and the "sequencing primer" 628A (CCC AAA GCC AGG CCG (SEQ ID NO: 12)) followed by digestion with Eco RI and KpnI.

A vector encoding the *Taq* gene (pLSM5 (SEQ ID NO: 3) producing the enzyme REM-T2 (SEQ ID NO: 4)) was digested with Eco RI and KpnI and purified. The inserts previously generated were then inserted into this vector. Ligation for insertion of the modified *Taq* gene was followed by transformation of DH5a cells followed by growth of individual colonies with assay for the DNA polymerase activity and the 5'-3' exonuclease activity.

EXAMPLE 3

Zone Mutagenesis of the *Taq* DNA Polymerase Gene - Treatment 3

Using PCR, we generated a *Taq* gene (REM-T2), which we cloned into the plasmid vector pUC18. See Figure 1. We amplified the N-terminal region of the *Taq* DNA polymerase gene for three consecutive PCR programs of 30 cycles each using the "reverse primer" for pUC18 (CAG GAA ACA GCT ATG ACC) and the "sequencing primer" 628A (CCC AAA GCC AGG CCG (SEQ ID NO: 12)). Inserts for the putative amino terminal region of the gene were generated by digestion of the PCR products with Eco RI and KpnI.

A vector encoding the *Taq* gene (pLSM5 (SEQ ID NO: 3) producing the enzyme REM-T2 (SEQ ID NO: 4)) was digested with Eco RI and KpnI and purified.

The inserts previously generated were then inserted into this vector. Ligation for insertion of the modified *Taq* gene was followed by transformation of DH5a cells followed by growth of individual colonies with assay for the DNA polymerase activity and the 5'-3' exonuclease activity.

5

EXAMPLE 4

Zone Mutagenesis of the *Taq* DNA Polymerase Gene - Treatment 4

Using PCR, we generated a *Taq* gene (REM-T2), which we cloned into the plasmid vector pUC18. See Figure 1. We incubated the plasmid DNA containing the *Taq* gene at pH 4.8 and 70°C for 15 minutes followed by neutralization to pH 8 with
10 50 mM Tris HCl. Inserts for the putative amino terminal region of the gene were generated by PCR using the "reverse primer" for pUC18 (CAG GAA ACA GCT ATG ACC (SEQ ID NO: 11)) and the "sequencing primer" 1155A (CAG GTC CCT GAG GGC (SEQ ID NO: 13)) and 5x concentration of dNTPs (0.75 mM) followed by digestion with Eco RI and BstXI.

15

A vector encoding the *Taq* gene (pLSM5 (SEQ ID NO: 3) producing the enzyme REM-T2 (SEQ ID NO: 4)) was digested with Eco RI and BstXI and purified. The inserts previously generated were then inserted into this vector. Ligation for insertion of the modified *Taq* gene was followed by transformation of DH5a cells followed by growth of individual colonies with assay for the DNA polymerase activity
20 and the 5'-3' exonuclease activity.

EXAMPLE 5**DNA Polymerase Activity Assay****Assay mixture:**

reaction volume: 0.3 ml

5 25 mM Tris-HCl (pH = 8.8)

4 mM MgCl₂

22 µg activated ssDNA (salmon sperm)

0.033 mM dNTP (each)

2 µCi [methyl-³H] thymidine 5' triphosphate

10 enzyme

Assay procedure:

The mixture was incubated at 75°C for 10 minutes. The reaction was stopped with 2 ml ice cold 10% TCA - 0.1 M sodium pyrophosphate. The tubes were then placed on ice for 10 minutes and the reaction volume filtered. The tube and filter
15 were washed three times with 2 ml of 10% TCA - 0.1 M sodium pyrophosphate. The filter was then washed with 10 ml 0.01 N HCl. Next the filters were dried at 120°C for 15 minutes. The dried filters were counted in 1 ml of Scintiverse.

The results are displayed in Table 1, *infra*.

EXAMPLE 6**5'-3' Exonuclease Activity Assay****Preparation of double stranded substrate with blunt ends and removal of 5' phosphate**

5 A Blue-Script plasmid was cut with HincII to produce one double stranded piece with blunt ends and treated with CIP (calf intestine phosphatase) to remove the 5' phosphate.

End-labeling of the 5' ends using [γ - 32 P]ATP

10 8 μ l plasmid and 4 μ l buffer were mixed with spermidine and 28 μ l distilled H₂O. The mixture was then heated to 70°C for 5 minutes and then chilled on ice for 2 minutes. 10 μ l kinase buffer with 1 μ l [γ - 32 P]ATP (about 10 mCi) and 2 μ l (20 units) of T4 polynucleotide kinase were added. Then the mixture was incubated for 30 minutes at 37°C. The reaction was stopped by adding 2 μ l 0.5 M EDTA. The enzyme was inactivated by incubating for 10 minutes at 70°C. The radioactive ATP was removed by washing 4 times (2 ml each) in Centricon 100. The final volume was about 50 μ l (38,000 cpm/ μ l).

5'-3' exonuclease assay

20 Assay conditions:

 reaction volume 50 μ l

 25 mM Tris HCl (8.8)

 4 mM Mg Cl₂

21

0.5 - 1 μ l labeled substrate

0.3 units of DNA polymerase

Samples were incubated at 50 - 55°C for 15, 30 or 60 minutes. The reaction was stopped with 0.3 ml 10% TCA. The sample was microfuged for 15 minutes at 4°C. 0.1 ml was sampled on filter paper. The filter paper was dried at 120°C for 15 minutes. Dried filters were counted in 1 ml of Scintiverse.

The assay results are presented in Table 1, *infra*.

EXAMPLE 7

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Sequencing Mutant Genes

Three mutants were chosen from those listed in Table 1 for low exonuclease activity. These were colony 18⁺ (the plasmid of which we designate pTarf2 (SEQ ID NO: 9)) and colony 20⁺ (the plasmid of which we designate pTarf3 (SEQ ID NO: 5)). A third mutant, pTarf5 (SEQ ID NO: 7), was obtained in a similar manner as in Example 4. pTarf3 (SEQ ID NO: 5) produces REM-T3 (SEQ ID NO: 6) and pTarf5 (SEQ ID NO: 7) produces REM-T5 (SEQ ID NO: 8). Bi-directional sequencing of the nucleic acid sequence of these mutants was conducted in the following manner: DNA sequence analysis was performed on alkaline-denatured double stranded plasmids. We used synthesized oligonucleotide primers (Fig. 3), [α -³⁵S]-dATP, and Sequenase® T7 DNA polymerase kit (United States Biochemical Corp.) according to the manufacturer's conditions. This method is based on the dideoxy chain termination reaction (Sanger, *Science* 214, 1205 (1981)).

The alterations found in the mutants are presented in Table 2. These alterations are of the pLSM5 (SEQ ID NO: 3) sequence, i.e., the pTarf2 (SEQ ID NO: 9), pTarf3 (SEQ ID NO: 5), and pTarf5 (SEQ ID NO: 7) sequences are the same as the pLSM5 (SEQ ID NO: 3) sequence except for the alterations listed in Table 2.

TABLE 1**Enzyme Activity Of New *Taq* Clones**

treatment	colony	polymerase act units/ μ l	5'-3' exonuclease activity % of REM-T2 (SEQ ID NO: 4)
1	1	0.132	87
	2	0.503	97
	3	0.053	14
	4	0.27	88
	5	0.098	82
	6	0.41	94
	7	0.255	95
2	8	0.106	74
1	1'	1.54	104
	2'	1.60	94
	3'	1.06	105
	4'	1.49	100
	5'	1.06	104
	6'	2.20	114
	7'	0.35	107
	8'	0.68	117

23

	9	0.74	94
	10	0.87	109
2	11	1.81	98
	12	1.22	95
5	13	1.68	110
	14	1.04	102
	15	0.84	101
	16	1.4	98
	17	0.15	104
10	18	1.77	24
	19	1.11	107
3	20	1.73	0
	21	0.018	6
	22	0.48	0
15	23	1.8	105
	24	0.83	94
	25	0.78	93

1 unit of polymerase activity = 10 nmoles of total nucleotides incorporated into acid insoluble form in 30 minutes at 75°C. Primed and unprimed colonies were obtained from cells transformed on different days.

TABLE 2*Alterations Relative to pLSM5 (SEQ ID NO: 3)*

plasmid	nucleotide position	amino acid position	codon change	amino acid change
p T a r f 2 (SEQ ID NO: 9)	337	73	TTC-CTC	Phe-Leu
pTarf3 (SEQ ID NO: 5)	193 504	25 128	CGC-TGC AAG-AAA	Arg-Cys Lys-Lys
p T a r f 5 (SEQ ID NO: 7)	341	74	CGC-CAC	Arg-His

EXAMPLE 8**Improved Processivity of the Modified *Taq* Polymerase**

Processivity of DNA synthesis by the modified *Taq* DNA polymerase (REM-T3) was assessed by several trials, with comparison to commercial enzymes and REM-T2. The method using the PCR protocol is novel.

Trial 1: Gel analysis of processivity by thermal stable DNA polymerases.

M13mp18 template (0.25 pmol/10 μ l) and 5' 32 P-labeled 17-mer (M13/pUC-40, BioLabs) (0.50 pmol/10 μ l) (calculated $t_m = 52^\circ\text{C}$) were annealed in 40 μ l of 10 mM Tris-HCl (pH 8.0), and 5 mM MgCl_2 . The mixture was incubated for 3 minutes at 90°C , 20 minutes at 42°C , and 15 minutes at room temperature. The reaction mixture was adjusted to 200 μM each of dNTP, 0.05% Tween 20 and Nonidet P-40, 10 mM Tris-HCl (pH 8.0), 50 mM KCl and 2.5 mM MgCl_2 , in a total

volume of 80 μ l, then incubated at 55°C for 2 minutes without enzyme. Next, 0.94 units of enzyme (AmpliTaq™ (Cetus), Stoffel Fragment(Cetus), REM-T2 or REM-T3)/10 μ l were added to start the reaction. Five μ l aliquots were removed from the reaction mixture at 0, 15, 30, 45 seconds, and 1, 2, and 5 minutes and added to 5 μ l of stop solution (1 mg/ml each of xylene cyanol and bromphenol blue, 10 mM EDTA in formamide). For gel analysis, 5 μ l were loaded onto a 6% wedge acrylamide/urea gel.

Figure 4 is a schematic depiction of the process and Figure 5 is an autoradiograph showing the results of trial 1.

10 Trial 2: Gel analysis of processivity by thermal stable DNA polymerases.

The same method was used as in Trial 1, except 0.22 units of polymerase/10 μ l of reaction mixture were added. In addition, smaller volumes were used for annealing (25 μ l) and reaction mixture (50 μ l).

For trials 1 and 2, the assayed polymerase activity of the AmpliTaq™ was lower than usual. It appears from the gels that the number of actual units of AmpliTaq™ used in the reaction may have been higher than estimated and, therefore, may not be comparable to the other reactions.

Figure 6 shows the results of trial 2. Note that when the amount of polymerase is limiting, REM-T2 (SEQ ID NO: 4) and REM-T3 (SEQ ID NO: 6) have processivities greater than that of the Stoffel fragment.

Trial 3: PCR analysis of processivity by thermal stable DNA polymerases.

The final volume of PCR reaction was 50 μ l. The buffer contained 67 mM Tris-HCl (pH 8.8), 16 mM $(\text{NH}_4)_2\text{SO}_4$, 10 mM beta mercaptoethanol, 2 mM MgCl_2 , 6.7 μ M EDTA, and 150 μ M each dNTP. There was an excess of template (0.02 pmol/10 μ l) and primers (each 10 pmol/10 μ l) over enzyme (0.04 units of polymerase/ 10 μ l) for each PCR reaction. The template was pLSM5 (SEQ ID NO: 3), a 5.1 kb plasmid containing Taq DNA polymerase gene and used for sequencing. For the 834-951 primer set, at least 102 nucleotides must be added to the primers to form the 117 base pair product, and for the 1564 -1937 primer set, at least 358 nucleotides must be added to the primer to form the 373 base pair product . The PCR program was 20 sec denaturation at 94°C, 30 sec annealing at 48°C, and 2 min extension at 72°C for 12 cycles.

Figure 7 is a schematic depiction of this process and Figure 8 shows is an autoradiograph showing the results.

Interpretation of Processivity Testing

Trials I and 2 are based on methodology similar to Innis et al., *Proc. Natl. Acad. Sci.* 85, 9436 (1988); Tabor et al., *J. Biol. Chem.* 262, 16212 (1987); and Wernette et al., *Biochem.* 27, 6046 (1988). The use of a fixed primer for synthesis under conditions of limiting enzyme activity and excess template/primer allows analysis of the length of extension of the primer with minimal chance for re-initiation. Thus, analysis of product size by polyacrylamide/urea gel measures primer extension as a unit event, or processivity of the polymerase (trials 1 and 2).

Trial 3 is based on a new approach. We reasoned that it would be possible to measure processivity under conditions of PCR. With limiting enzyme concentration and excess primer/template concentration, the probability of re-initiation on a partially extended primer in PCR cycles is very low. Therefore, the length of the observed product (resulting from the complete extension of a primer through the opposing primer) is a measure of processivity. We found that 12 cycles results in sufficient yield to detect products with ethidium bromide on agarose gel. By varying the distance between primers we can determine a processivity range. AmpliTaq™, REM-T2, and REM-T3 have a processivity of at least 105 nucleotides, but less than 358 nucleotides. Stoffel Fragment, on the other hand has a processivity of less than 105 nucleotides.

Figure 8 compares the ability of four polymerases to extend a primer 105 nucleotides (Lanes 1-4) or 358 nucleotides (Lanes 5-8) under PCR conditions of excess DNA template (0.02 pmol/10 μ l of reaction) and primer (10 pmol/10 μ l of reaction) and limited polymerase units (0.04 units of polymerase/10 μ l reaction). PCR products are shown on a 3% NuSieve gel. AmpliTaq™ is in lanes 1 and 5, Stoffel Fragment is in lanes 2 and 6, REM-T2 in lanes 3 and 7, and REM-T3 in lanes 4 and 8. Marker lane has ϕ x 174/Hae III.

It is evident from an examination of Figures 6, 7, and 8 that REM-T3 (SEQ ID NO: 6) has a processivity equal to or better than AmpliTaq™, and much better than the Stoffel fragment. This result demonstrates that the full length polypeptide of the modified *Taq* enzyme confers superior processivity compared to the truncated peptide of the Stoffel enzyme.

EXAMPLE 9**Misincorporation Rate for Modified *Taq* DNA Polymerases**

Information already published by Barnes, *Gene* 112, 29-35 (1992) indicates that *Taq*
5 DNA polymerase which has had the N-terminal region containing the 5' exonuclease
domain removed has a diminished misincorporation rate. The information available
indicates that such a modified *Taq* DNA polymerase has a two-fold lower
misincorporation rate than native *Taq* DNA polymerase. Since the evidence
presented by Barnes leads to the conclusion that the misincorporation by the *Taq*
10 DNA polymerase is lowered in the absence of the exonuclease activity, we are
motivated to measure the misincorporation rate of the modified *Taq* DNA
polymerases described herein.

The assessment of misincorporation is done by several methodologies:

1. The methodology of Barnes uses a specially constructed plasmid with
15 a flanking selectable marker, based on identification of *lacZ* as an indicator gene.
Scoring for misincorporation in the *lac* gene is by the familiar blue/white test on an
indicator dye (XGal). Testing for misincorporation is performed by inserting the
plasmids into an indicator bacterial strain following PCR reactions *in vitro*.
2. The methodology of Tindall and Kunkel, *Biochemistry* 21, 6008-6013
20 (1988) monitors the fidelity of *in vitro* DNA synthesis using the *lacZ* gene for a
complementation in a plasmid derived from M13 bacteriophage. Measurement of
misincorporation is based on the blue/white test for *lacZ* function using an indicator
dye in the plate. The plasmid derivative contains an open single-stranded gap region

of 390 nucleotides. This construction allows measurement of the forward mutation rate, or the substantially lower reversion mutation rate for any specific misincorporation constructed. The results found by Kunkel and coworkers, indicate that the native *Taq* DNA polymerase has a base substitution error rate of approximately 1/9000 nucleotides polymerized.

The processivity of our modified *Taq* DNA polymerase is much higher than the processivity of the truncated proteolytic fragment, and since the DNA polymerase literature indicates that misincorporation correlates with re-initiation, our misincorporation rate is considerably improved relative to native *Taq* DNA polymerase.

30

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Moses M.D., Robb E.

(ii) TITLE OF INVENTION: Modified Thermo-Resistant DNA Polymerases

(iii) NUMBER OF SEQUENCES: 15

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Allegretti & Witcoff

(B) STREET: 10 South Wacker Drive

(C) CITY: Chicago

(D) STATE: IL

(E) COUNTRY: USA

(F) ZIP: 60606

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Greenfield Ph.D., Michael S.

(B) REGISTRATION NUMBER: 37,142

(C) REFERENCE/DOCKET NUMBER: 93,413

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (312)715-1000

(B) TELEFAX: (312)715-1234

(2) INFORMATION FOR SEQ ID NO::1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2626 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Thermus aquaticus

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 121..2619

(ix) FEATURE:

31

(A) NAME/KEY: mat_peptide
(B) LOCATION: 121..2616

(ix) FEATURE:

(A) NAME/KEY: -
(B) LOCATION: 1..2625
(D) OTHER INFORMATION: /note= "Native Taq DNA Polymerase
nucleotide sequence."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO::1:

5	AAGCTCAGAT CTACCTGCCT GAGGGGCTCC GGTTCAGCT GGCCCTTCCC GAGGGGGAGA	60
15	GGGAGGCGTT TCTAAAAGCC CTTCAGGAAG CTACCCGGGG GCGGGTGGTG GAAGGGTAAC	120
	ATG AGG GGG ATG CTG CCC CTC TTT GAG CCC AAG GGC CGG GTC CTC CTG	168
	Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu	
20	1 5 10 15	
	GTG GAC GGC CAC CAC CTG GCC TAC CGC ACC TTC CAC GCC CTG AAG GGC	216
	Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly	
	20 25 30	
25	CTC ACC ACC AGC CGG GGG GAG CCG GTG CAG GCG GTC TAC GGC TTC GCC	264
	Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala	
	35 40 45	
30	AAG AGC CTC CTC AAG GCC CTC AAG GAG GAC GGG GAC GCG GTG ATC GTG	312
	Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val	
	50 55 60	
35	GTC TTT GAC GCC AAG GCC CCC TCC TTC CGC CAC GAG GCC TAC GGG GGG	360
	Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly	
	65 70 75 80	
40	TAC AAG GCG GGC CGG GCC CCC ACG CCG GAG GAC TTT CCC CGG CAA CTC	408
	Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu	
	85 90 95	
	GCC CTC ATC AAG GAG CTG GTG GAC CTC CTG GGG CTG GCG CGC CTC GAG	456
	Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu	
	100 105 110	
45	GTC CCG GGC TAC GAG GCG GAC GAC GTC CTG GCC AGC CTG GCC AAG AAG	504
	Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys	
	115 120 125	
50	GCG GAA AAG GAG GGC TAC GAG GTC CGC ATC CTC ACC GCC GAC AAA GAC	552
	Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp	
	130 135 140	
55	CTT TAC CAG CTC CTT TCC GAC CGC ATC CAC GTC CTC CAC CCC GAG GGG	600
	Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly	
	145 150 155 160	
	TAC CTC ATC ACC CCG GCC TGG CTT TGG GAA AA TAC GGC CTG AGG CCC	648
	Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro	
	165 170 175	
60	GAC CAG TGG GCC GAC TAC CGG GCC CTG ACC GGG GAC GAG TCC GAC AAC	696
	Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn	
	180 185 190	

32

	CTT CCC GGG GTC AAG GGC ATC GGG GAG AAG ACG GCG AGG AAG CTT CTG Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu	744
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	245 250 255	
20	GAC TTC GCC AAA AGG CGG GAG CCC GAC CGG GAG AGG CTT AGG GCC TTT Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Phe	936
	260 265 270	
25	CTG GAG AGG CTT GAG TTT GGC AGC CTC CTC CAC GAG TTC GGC CTT CTG Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu Leu	984
	275 280 285	
30	GAA AGC CCC AAG GCC CTG GAG GAG GCC CCC TGG CCC CCG CCG GAA GGG Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly	1032
	290 295 300	
35	GCC TTC GTG GGC TTT GTG CTT TCC CGC AAG GAG CCC ATG TGG GCC GAT Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp	1080
	305 310 315 320	
40	CTT CTG GCC CTG GCC GCC GCC AGG GGG GGC CGG GTC CAC CGG GCC CCC Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro	1128
	325 330 335	
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	340 345 350	
50	GCC AAA GAC CTG AGC GTT CTG GCC CTG AGG GAA GGC CTT GGC CTC CCG Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro	1224
	355 360 365	
55	CCC GGC GAC GAC CCC ATG CTC CTC GCC TAC CTC CTG GAC CCT TCC AAC Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn	1272
	370 375 380	
60	ACC ACC CCC GAG GGG GTG GCC CGG CGC TAC GGC GGG GAG TGG ACG GAG Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu	1320
	385 390 395 400	
65	GAG GCG GGG GAG CGG GCC GCC CTT TCC GAG AGG CTC TTC GCC AAC CTG Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu	1368
	405 410 415	
70	TGG GGG AGG CTT GAG GGG GAG GAG AGG CTC CTT TGG CTT TAC CGG GAG Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu	1416
	420 425 430	
75	GTG GAG AGG CCC CTT TCC GCT GTC CTG GCC CAC ATG GAG GCC ACG GGG Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly	1464
	435 440 445	

33

	GTG CGC CTG GAC GTG GCC TAT CTC AGG GCC TTG TCC CTG GAG GTG GCC Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala 450 455 460	1512
5	GAG GAG ATC GCC CGC CTC GAG GCC GAG GTC TTC CGC CTG GCC GGC CAC Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His 465 470 475 480	1560
10	CCC TTC AAC CTC AAC TCC CGG GAC CAG CTG GAA AGG GTC CTC TTT GAC Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp 485 490 495	1608
15	GAG CTA GGG CTT CCC GCC ATC GGC AAG ACG GAG AAG ACC GGC AAG CGC Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg 500 505 510	1656
20	TCC ACC AGC GCC GCC GTC CTG GAG GCC CTC CGC GAG GCC CAC CCC ATC Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile 515 520 525	1704
25	GTG GAG AAG ATC CTG CAG TAC CGG GAG CTC ACC AAG CTG AAG AGC ACC Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr 530 535 540	1752
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35	CAC ACC CGC TTC AAC CAG ACG GCC ACG GCC ACG GGC AGG CTA AGT AGC His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser 565 570 575	1848
40	TCC GAT CCC AAC CTC CAG AAC ATC CCC GTC CGC ACC CCG CTT GGG CAG Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln 580 585 590	1896
45	AGG ATC CGC CGG GCC TTC ATC GCC GAG GAG GGG TGG CTA TTG GTG GCC Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala 595 600 605	1944
50	CTG GAC TAT AGC CAG ATA GAG CTC AGG GTG CTG GCC CAC CTC TCC GGC Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly 610 615 620	1992
55	GAC GAG AAC CTG ATC CGG GTC TTC CAG GAG GGG CGG GAC ATC CAC ACG Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr 625 630 635 640	2040
60	GAG ACC GCC AGC TGG ATG TTC GGC GTC CCC CGG GAG GCC GTG GAC CCC Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro 645 650 655	2088
65	CTG ATG CGC CGG GCG GCC AAG ACC ATC AAC TTC GGG GTC CTC TAC GGC Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly 660 665 670	2136
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34

5	GCC TGG ATT GAG AAG ACC CTG GAG GAG GGC AGG AGG CCG GGG TAC GTG Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val 705 710 715 720	2280
10	GAG ACC CTC TTC GGC CGC CGC CGC TAC GTG CCA GAC CTA GAG GCC CCG Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg 725 730 735	2328
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25	TTC CCC AGG CTG GAG GAA ATG GGG GCC AGG ATG CTC CTT CAG GTC CAC Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His 770 775 780	2472
30	GAC GAG CTG GTC CTC GAG GCC CCA AAA GAG AGG GCG GAG GCC GTG GCC Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala 785 790 795 800	2520
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40	CTG GAG GTG GAG GTG GGG ATA GGG GAG GAC TGG CTC TCC GCC AAG GAG Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu 820 825 830	2616
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(2) INFORMATION FOR SEQ ID NO::2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 832 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO::2:

Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu 1 5 10 15	
Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly 20 25 30	
Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala 35 40 45	
Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val 50 55 60	
Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly 65 70 75 80	

35

Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu
 85 90 95
 5 Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu
 100 105 110
 Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys
 115 120 125
 10 Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp
 130 135 140
 Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly
 145 150 155 160
 15 Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro
 165 170 175
 Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn
 180 185 190
 20 Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu
 195 200 205
 25 Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu
 210 215 220
 Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys
 225 230 235 240
 30 Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val
 245 250 255
 Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Phe
 260 265 270
 35 Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu Leu
 275 280 285
 40 Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly
 290 295 300
 Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp
 305 310 315 320
 45 Leu Leu Ala Leu Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro
 325 330 335
 50 Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu
 340 345 350
 Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro
 355 360 365
 55 Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn
 370 375 380
 Thr Thr Pro Glu ly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu
 385 390 395 400
 60 Glu Ala ly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu
 405 410 415

36

Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu
 420 425 430
 5 Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly
 435 440 445
 Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala
 450 455 460
 10 Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His
 465 470 475 480
 Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp
 485 490 495
 15 Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg
 500 505 510
 Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile
 515 520 525
 20 Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr
 530 535 540
 25 Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu
 545 550 555 560
 His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser
 565 570 575
 30 Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln
 580 585 590
 Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala
 595 600 605
 35 Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly
 610 615 620
 40 Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr
 625 630 635 640
 Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro
 645 650 655
 45 Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly
 660 665 670
 Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu
 675 680 685
 50 Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg
 690 695 700
 55 Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val
 705 710 715 720
 Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg
 725 730 735
 60 Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro
 740 745 750

37

Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu
 755 760 765

5 Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His
 770 775 780

Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala
 785 790 795 800

10 Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro
 805 810 815

Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu
 820 825 830

15

(2) INFORMATION FOR SEQ ID NO::3:

- 20 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 2626 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- 25 (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- 30 (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: *Thermus aquaticus*
- 35 (ix) FEATURE:
 (A) NAME/KEY: mutation
 (B) LOCATION: replace(89, "g")
 (D) OTHER INFORMATION: /note= "This mutation results in a
 nucleotide alteration at position 89 of the native Taq DNA
 polymerase nucleotide sequence of C to G."
- 40 (ix) FEATURE:
 (A) NAME/KEY: mutation
 (B) LOCATION: replace(934, "a")
 (D) OTHER INFORMATION: /note= "This mutation results in a
 nucleotide alteration at position 934 of the native Taq
 DNA polymerase nucleotide sequence of T to A. This
 results in an amino acid change of Phe to Ile."
- 45 (ix) FEATURE:
 (A) NAME/KEY: mutation
 (B) LOCATION: replace(962, "c")
 (D) OTHER INFORMATION: /note= "This mutation results in a
 nucleotide alteration at position 962 of the native Taq
 DNA polymerase nucleotide sequence of T to C. This
 results in an amino acid change of Leu to Pro."
- 50 (ix) FEATURE:
 (A) NAME/KEY: mutation
 (B) LOCATION: replace(2535, "a")
 (D) OTHER INFORMATION: /note= "This mutation results in a
 nucleotide alteration at position 2535 of the native Taq
 DNA polymerase nucleotide sequence of G to A. This
 mutation is conservative."
- 55
- 60

38

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 121..2619

5

(ix) FEATURE:

(A) NAME/KEY: mat_peptide

(B) LOCATION: 121..2616

10

(ix) FEATURE:

(A) NAME/KEY: -

(B) LOCATION: 1..2619

(D) OTHER INFORMATION: /note= "pLSM5"

15

(x1) SEQUENCE DESCRIPTION: SEQ ID NO::3:

	AAGCTCAGAT CTACCTGCCT GAGGGCGTCC GGTTCAGCT GGCCCTTCCC GAGGGGGAGA	60
	GGGAGGCGTT TCTAAAAGCC CTTCAGGAGG CTACCCGGGG GCGGGTGGTG GAAGGGTAAC	120
20	ATG AGG GGG ATG CTG CCC CTC TTT GAG CCC AAG GGC CGG GTC CTC CTG Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu 1 5 10 15	168
25	GTG GAC GGC CAC CAC CTG GCC TAC CGC ACC TTC CAC GCC CTG AAG GGC Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly 20 25 30	216
30	CTC ACC ACC AGC CGG GGG GAG CCG GTG CAG GCG GTC TAC GGC TTC GCC Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala 35 40 45	264
35	AAG AGC CTC CTC AAG GCC CTC AAG GAG GAC GGG GAC GCG GTG ATC GTG Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val 50 55 60	312
40	GTC TTT GAC GCC AAG GCC CCC TCC TTC CGC CAC GAG GCC TAC GGG GGG Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly 65 70 75 80	360
45	TAC AAG GCG GGC CGG GCC CCC ACG COG GAG GAC TTT CCC CGG CAA CTC Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu 85 90 95	408
50	GCC CTC ATC AAG GAG CTG GTG GAC CTC CTG GGG CTG GCG CGC CTC GAG Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu 100 105 110	456
55	GTC CCG GGC TAC GAG GCG GAC GAC GTC CTG GCC AGC CTG GCC AAG AAG Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys 115 120 125	504
60	GCG GAA AAG GAG GGC TAC GAG GTC CGC ATC CTC ACC GCC GAC AAA GAC Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp 130 135 140	552
	CTT TAC CAG CTC CTT TCC GAC CGC ATC CAC GTC CTC CAC CCC GAG GGG Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu ly 145 150 155 160	600
	TAC CTC ATC ACC CCG GCC TGG CTT TGG GAA AAG TAC GGC CTG AGG CCC Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro 165 170 175	648

39

	GAC CAG TGG GCC GAC TAC CGG GCC CTG ACC GGG GAC GAG TCC GAC AAC Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn 180 185 190	696
5	CTT CCC GGG GTC AAG GGC ATC GGG GAG AAG ACG GCG AGG AAG CTT CTG Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu 195 200 205	744
10	GAG GAG TGG GGG AGC CTG GAA GCC CTC CTC AAG AAC CTG GAC CGG CTG Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu 210 215 220	792
15	AAG CCC GCC ATC CGG GAG AAG ATC CTG GCC CAC ATG GAC GAT CTG AAG Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys 225 230 235 240	840
20	CTC TCC TGG GAC CTG GCC AAG GTG CGC ACC GAC CTG CCC CTG GAG GTG Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val 245 250 255	888
	GAC TTC GCC AAA AGG CGG GAG CCC GAC CGG GAG AGG CTT AGG GCC ATT Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Ile 260 265 270	936
25	CTG GAG AGG CTT GAG TTT GGC AGC CCC CTC CAC GAG TTC GGC CTT CTG Leu Glu Arg Leu Glu Phe Gly Ser Pro Leu His Glu Phe Gly Leu Leu 275 280 285	984
30	GAA AGC CCC AAG GCC CTG GAG GAG GCC CCC TGG CCC CCG CCG GAA GGG Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly 290 295 300	1032
35	GCC TTC GTG GGC TTT GTG CTT TCC CGC AAG GAG CCC ATG TGG GCC GAT Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp 305 310 315 320	1080
40	CTT CTG GCC CTG GCC GCC GCC AGG GGG GGC CGG GTC CAC CGG GCC CCC Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro 325 330 335	1128
	GAG CCT TAT AAA GCC CTC AGG GAC CTG AAG GAG GCG CGG GGG CTT CTC Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu 340 345 350	1176
45	GCC AAA GAC CTG AGC GTT CTG GCC CTG AGG GAA GGC CTT GGC CTC CCG Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro 355 360 365	1224
50	CCC GGC GAC GAC CCC ATG CTC CTC GCC TAC CTC CTG GAC CCT TCC AAC Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn 370 375 380	1272
55	ACC ACC CCC GAG GGG GTG GCC CGG CGC TAC GGC GGG GAG TGG ACG GAG Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu 385 390 395 400	1320
	GAG GCG GGG GAG CGG GCC GCC CTT TCC GAG AGG CTC TTC GCC AAC CTG Glu Ala Gly lu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu 405 410 415	1368
60	TGG GGG AGG CTT GAG GGG GAG GAG AGG CTC CTT TGG CTT TAC CGG GAG Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu 420 425 430	1416

40

	GTG GAG AGG CCC CTT TCC GCT GTC CTG GCC CAC ATG GAG GCC ACG GGG	1464
	Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly	
	435 440 445	
5	GTG CGC CTG GAC GTG GCC TAT CTC AGG GCC TTG TCC CTG GAG GTG GCC	1512
	Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala	
	450 455 460	
10	GAG GAG ATC GCC CGC CTC GAG GCC GAG GTC TTC CGC CTG GCC GGC CAC	1560
	Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His	
	465 470 475 480	
15	CCC TTC AAC CTC AAC TCC CGG GAC CAG CTG GAA AGG GTC CTC TTT GAC	1608
	Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp	
	485 490 495	
20	GAG CTA GGG CTT CCC GCC ATC GGC AAG ACG GAG AAG ACC GGC AAG CGC	1656
	Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg	
	500 505 510	
25	TCC ACC AGC GCC GCC GTC CTG GAG GCC CTC CGC GAG GCC CAC CCC ATC	1704
	Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile	
	515 520 525	
30	GTG GAG AAG ATC CTG CAG TAC CGG GAG CTC ACC AAG CTG AAG AGC ACC	1752
	Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr	
	530 535 540	
35	TAC ATT GAC CCC TTG CCG GAC CTC ATC CAC CCC AGG ACG GGC CGC CTC	1800
	Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu	
	545 550 555 560	
40	CAC ACC CGC TTC AAC CAG ACG GCC ACG GCC ACG GGC AGG CTA AGT AGC	1848
	His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser	
	565 570 575	
45	TCC GAT CCC AAC CTC CAG AAC ATC CCC GTC CGC ACC CCG CTT GGC CAG	1896
	Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln	
	580 585 590	
50	AGG ATC CGC CGG GCC TTC ATC GCC GAG GAG GGG TGG CTA TTG GTG GCC	1944
	Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala	
	595 600 605	
55	CTG GAC TAT AGC CAG ATA GAG CTC AGG GTG CTG GCC CAC CTC TCC GGC	1992
	Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly	
	610 615 620	
60	GAC GAG AAC CTG ATC CGG GTC TTC CAG GAG GGG CGG GAC ATC CAC ACG	2040
	Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr	
	625 630 635 640	
65	GAG ACC GCC AGC TGG ATG TTC GGC GTC CCC CGG GAG GCC GTG GAC CCC	2088
	Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro	
	645 650 655	
70	CTG ATG CGC CGG GCG GCC AAG ACC ATC AAC TTC GGG GTC CTC TAC GGC	2136
	Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe ly Val Leu Tyr Gly	
	660 665 670	
75	ATG TCG GCC CAC CGC CTC TCC CAG GAG CTA GCC ATC CCT TAC GAG GAG	2184
	Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu lu	
	675 680 685	

41

	GCC CAG GCC TTC ATT GAG CGC TAC TTT CAG AGC TTC CCC AAG GTG CGG	2232
	Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg	
	690 695 700	
5	GCC TGG ATT GAG AAG ACC CTG GAG GAG GGC AGG AGG CGG GGG TAC GTG	2280
	Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val	
	705 710 715 720	
10	GAG ACC CTC TTC GGC CGC CGC CGC TAC GTG CCA GAC CTA GAG GCC CGG	2328
	Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg	
	725 730 735	
15	GTG AAG AGC GTG CGG GAG GCG GCC GAG CGC ATG GCC TTC AAC ATG CCC	2376
	Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro	
	740 745 750	
20	GTC CAG GGC ACC GCC GCC GAC CTC ATG AAG CTG GCT ATG GTG AAG CTC	2424
	Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu	
	755 760 765	
25	TTC CCC AGG CTG GAG GAA ATG GGG GCC AGG ATG CTC CTT CAG GTC CAC	2472
	Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His	
	770 775 780	
30	GAC GAG CTG GTC CTC GAG GCC CCA AAA GAG AGG GCG GAG GCC GTG GCC	2520
	Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala	
	785 790 795 800	
35	CGG CTG GCC AAG GAA GTC ATG GAG GGG GTG TAT CCC CTG GCC GTG CCC	2568
	Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro	
	805 810 815	
40	CTG GAG GTG GAG GTG GGG ATA GGG GAG GAC TGG CTC TCC GCC AAG GAG	2616
	Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu	
	820 825 830	
45	TGATACCACC	2626
50	(2) INFORMATION FOR SEQ ID NO::4:	
	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 832 amino acids	
	(B) TYPE: amino acid	
	(D) TOPOLOGY: linear	
55	(ii) MOLECULE TYPE: protein	
60	(xi) SEQUENCE DESCRIPTION: SEQ ID NO::4:	
	Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu	
	1 5 10 15	
	Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly	
	20 25 30	
	Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala	
	35 40 45	
	Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val	
	50 55 60	

42

Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly
 65 70 75 80
 Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu
 5 85 90 95
 Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu
 100 105 110
 Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys
 10 115 120 125
 Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp
 130 135 140
 Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly
 145 150 155 160
 Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro
 165 170 175
 Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn
 180 185 190
 Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu
 195 200 205
 Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu
 210 215 220
 Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys
 225 230 235 240
 Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val
 245 250 255
 Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Ile
 260 265 270
 Leu Glu Arg Leu Glu Phe Gly Ser Pro Leu His Glu Phe Gly Leu Leu
 275 280 285
 Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly
 290 295 300
 Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp
 305 310 315 320
 Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro
 325 330 335
 Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu
 340 345 350
 Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro
 355 360 365
 Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn
 370 375 380
 Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu
 385 390 395 400

43

Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu
 405 410 415
 5 Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu
 420 425 430
 Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly
 435 440 445
 10 Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala
 450 455 460
 Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His
 465 470 475 480
 15 Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp
 485 490 495
 20 Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg
 500 505 510
 Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile
 515 520 525
 25 Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr
 530 535 540
 Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu
 545 550 555 560
 30 His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser
 565 570 575
 35 Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln
 580 585 590
 Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala
 595 600 605
 40 Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly
 610 615 620
 Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr
 625 630 635 640
 45 Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro
 645 650 655
 50 Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly
 660 665 670
 Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu
 675 680 685
 55 Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg
 690 695 700
 Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val
 705 710 715 720
 60 Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg
 725 730 735

44

Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro
 740 745 750

5 Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu
 755 760 765

Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His
 770 775 780

10 Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala
 785 790 795 800

Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro
 805 810 815

15 Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu
 820 825 830

20 (2) INFORMATION FOR SEQ ID NO::5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2626 base pairs
 (B) TYPE: nucleic acid
 25 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

30 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- 35 (A) ORGANISM: *Thermus aquaticus*

(ix) FEATURE:

- (A) NAME/KEY: mutation
 (B) LOCATION: replace(89, "g")
 40 (D) OTHER INFORMATION: /note= "This mutation results in a
 nucleotide alteration at position 89 of the native Taq DNA
 polymerase nucleotide sequence of C to G."

(ix) FEATURE:

- 45 (A) NAME/KEY: mutation
 (B) LOCATION: replace(934, "a")
 (D) OTHER INFORMATION: /note= "This mutation results in a
 nucleotide alteration at position 934 of the native Taq
 DNA polymerase nucleotide sequence of T to A. This
 50 results in an amino acid change of Phe to Ile."

(ix) FEATURE:

- 55 (A) NAME/KEY: mutation
 (B) LOCATION: replace(962, "c")
 (D) OTHER INFORMATION: /note= "This mutation results in a
 nucleotide alteration at position 962 of the native Taq
 DNA polymerase nucleotide sequence of T to C. This
 results in an amino acid change of Leu to Pro."

60

(ix) FEATURE:

- (A) NAME/KEY: mutation
 (B) LOCATION: replace(2535, "a")
 (D) OTHER INFORMATION: /note= "This mutation results in a

nucleotide alteration at position 2535 of the native Taq DNA polymerase nucleotide sequence of G to A. This mutation is conservative."

5

(ix) FEATURE:

(A) NAME/KEY: mutation

(B) LOCATION: replace(193, "t")

10

(D) OTHER INFORMATION: /note= "This mutation changes the nucleotide at position 193 of the native Taq DNA polymerase from C to T, resulting in an amino acid change of Arg to Cys."

(ix) FEATURE:

15

(A) NAME/KEY: mutation

(B) LOCATION: replace(504, "a")

(D) OTHER INFORMATION: /note= "This mutation changes the nucleotide at position 504 of the native Taq DNA polymerase from G to A, which is conservative in nature."

20

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 121..2619

(ix) FEATURE:

25

(A) NAME/KEY: mat_peptide

(B) LOCATION: 121..2616

(ix) FEATURE:

30

(A) NAME/KEY: -

(B) LOCATION: 1..2619

(D) OTHER INFORMATION: /note= "pTarf3"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO::5:

35

AAGCTCAGAT CTACCTGCCT GAGGGCGTCC GGTTCAGCT GGCCCTTCCC GAGGGGGAGA 60

GGGAGGCGTT TCTAAAAGCC CTTCAGGAGG CTACCCGGGG GCGGGTGGTG GAAGGGTAAC 120

40

ATG AGG GGG ATG CTG CCC CTC TTT GAG CCC AAG GGC CGG GTC CTC CTG 168

Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu
1 5 10 15

45

GTG GAC GGC CAC CAC CTG GCC TAC TGC ACC TTC CAC GCC CTG AAG GGC 216

Val Asp Gly His His Leu Ala Tyr Cys Thr Phe His Ala Leu Lys Gly
20 25 30

50

CTC ACC ACC AGC CGG GGG GAG CCG GTG CAG GCG GTC TAC GGC TTC GCC 264

Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala
35 40 45

55

AAG AGC CTC CTC AAG GCC CTC AAG GAG GAC GGG GAC GCG GTG ATC GTG 312

Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val
50 55 60

GTC TTT GAC GCC AAG GCC CCC TCC TTC CGC CAC GAG GCC TAC GGG GGG 360

Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly
65 70 75 80

60

TAC AAG GCG GGC CGG GCC CCC ACG CCG GAG GAC TTT CCC CGG CAA CTC 408

Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu
85 90 95

46

	GCC CTC ATC AAG GAG CTG GTG GAC CTC CTG GGG CTG GCG CGC CTC GAG	456
	Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu	
	100 105 110	
5	GTC CCG GGC TAC GAG GCG GAC GAC GTC CTG GCC AGC CTG GCC AAG AAA	504
	Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys	
	115 120 125	
10	GCG GAA AAG GAG GGC TAC GAG GTC CGC ATC CTC ACC GCC GAC AAA GAC	552
	Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp	
	130 135 140	
15	CTT TAC CAG CTC CTT TCC GAC CGC ATC CAC GTC CTC CAC CCC GAG GGG	600
	Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly	
	145 150 155 160	
20	TAC CTC ATC ACC CCG GCC TGG CTT TGG GAA AAG TAC GGC CTG AGG CCC	648
	Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro	
	165 170 175	
25	GAC CAG TGG GCC GAC TAC CGG GCC CTG ACC GGG GAC GAG TCC GAC AAC	696
	Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn	
	180 185 190	
30	CTT CCC GGG GTC AAG GGC ATC GGG GAG AAG ACG GCG AGG AAG CTT CTG	744
	Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu	
	195 200 205	
35	GAG GAG TGG GGG AGC CTG GAA GCC CTC CTC AAG AAC CTG GAC CGG CTG	792
	Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu	
	210 215 220	
40	AAG CCC GCC ATC CGG GAG AAG ATC CTG GCC CAC ATG GAC GAT CTG AAG	840
	Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys	
	225 230 235 240	
45	CTC TCC TGG GAC CTG GCC AAG GTG CGC ACC GAC CTG CCC CTG GAG GTG	888
	Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val	
	245 250 255	
50	GAC TTC GCC AAA AGG CGG GAG CCC GAC CGG GAG AGG CTT AGG GCC ATT	936
	Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Ile	
	260 265 270	
55	CTG GAG AGG CTT GAG TTT GGC AGC CCC CTC CAC GAG TTC GGC CTT CTG	984
	Leu Glu Arg Leu Glu Phe Gly Ser Pro Leu His Glu Phe Gly Leu Leu	
	275 280 285	
60	GAA AGC CCC AAG GCC CTG GAG GAG GCC CCC TGG CCC CCG CCG GAA GGG	1032
	Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly	
	290 295 300	
65	GCC TTC GTG GGC TTT GTG CTT TCC CGC AAG GAG CCC ATG TGG GCC GAT	1080
	Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp	
	305 310 315 320	
70	CTT CTG GCC CTG GCC GCC GCC AGG GGG GGC CGG GTC CAC CGG GCC CCC	1128
	Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro	
	325 330 335	
75	GAG CCT TAT AAA GCC CTC AGG GAC CTG AAG GAG GCG CGG GGG CTT CTC	1176
	Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu	
	340 345 350	

47

	GCC AAA GAC CTG AGC GTT CTG GCC CTG AGG GAA GGC CTT GGC CTC CCG Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro 355 360 365	1224
5	CCC GGC GAC GAC CCC ATG CTC CTC GCC TAC CTC CTG GAC CCT TCC AAC Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn 370 375 380	1272
10	ACC ACC CCC GAG GGG GTG GCC CGG CGC TAC GGC GGG GAG TGG ACG GAG Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu 385 390 395 400	1320
15	GAG GCG GGG GAG CGG GCC GCC CTT TCC GAG AGG CTC TTC GCC AAC CTG Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu 405 410 415	1368
20	TGG GGG AGG CTT GAG GGG GAG GAG AGG CTC CTT TGG CTT TAC CGG GAG Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu 420 425 430	1416
25	GTG GAG AGG CCC CTT TCC GCT GTC CTG GCC CAC ATG GAG GCC ACG GGC Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly 435 440 445	1464
30	GTG CGC CTG GAC GTG GCC TAT CTC AGG GCC TTG TCC CTG GAG GTG GCC Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala 450 455 460	1512
35	GAG GAG ATC GCC CGC CTC GAG GCC GAG GTC TTC CGC CTG GCC GGC CAC Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His 465 470 475 480	1560
40	CCC TTC AAC CTC AAC TCC CGG GAC CAG CTG GAA AGG GTC CTC TTT GAC Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp 485 490 495	1608
45	GAG CTA GGG CTT CCC GCC ATC GGC AAG ACG GAG AAG ACC GGC AAG CGC Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg 500 505 510	1656
50	TCC ACC AGC GCC GCC GTC CTG GAG GCC CTC CGC GAG GCC CAC CCC ATC Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile 515 520 525	1704
55	GTG GAG AAG ATC CTG CAG TAC CGG GAG CTC ACC AAG CTG AAG AGC ACC Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr 530 535 540	1752
60	TAC ATT GAC CCC TTG CCG GAC CTC ATC CAC CCC AGG ACG GGC CGC CTC Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu 545 550 555 560	1800
65	CAC ACC CGC TTC AAC CAG ACG GCC ACG GCC ACG GGC AGG CTA AGT AGC His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser 565 570 575	1848
70	TCC GAT CCC AAC CTC CAG AAC ATC CCC GTC CGC ACC CCG CTT GGG CAG Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln 580 585 590	1896
75	AGG ATC CGC CGG GCC TTC ATC GCC GAG GAG GGG TGG CTA TTG GTG GCC Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala 595 600 605	1944

48

	CTG GAC TAT AGC CAG ATA GAG CTC AGG GTG CTG GCC CAC CTC TCC GGC Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly 610 615 620	1992
5	GAC GAG AAC CTG ATC CGG GTC TTC CAG GAG GGG CGG GAC ATC CAC ACG Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr 625 630 635 640	2040
10	GAG ACC GCC AGC TGG ATG TTC GGC GTC CCC CGG GAG GCC GTG GAC CCC Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro 645 650 655	2088
15	CTG ATG CGC CGG GCG GCC AAG ACC ATC AAC TTC GGG GTC CTC TAC GGC Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly 660 665 670	2136
20	ATG TCG GCC CAC CGC CTC TCC CAG GAG CTA GCC ATC CCT TAC GAG GAG Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu 675 680 685	2184
	GCC CAG GCC TTC ATT GAG CGC TAC TTT CAG AGC TTC CCC AAG GTG CGG Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg 690 695 700	2232
25	GCC TGG ATT GAG AAG ACC CTG GAG GAG GGC AGG AGG CGG GGG TAC GTG Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val 705 710 715 720	2280
30	GAG ACC CTC TTC GGC CGC CGC CGC TAC GTG CCA GAC CTA GAG GCC CGG Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg 725 730 735	2328
35	GTG AAG AGC GTG CGG GAG GCG GCC GAG CGC ATG GCC TTC AAC ATG CCC Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro 740 745 750	2376
40	GTC CAG GGC ACC GCC GCC GAC CTC ATG AAG CTG GCT ATG GTG AAG CTC Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu 755 760 765	2424
	TTC CCC AGG CTG GAG GAA ATG GGG GCC AGG ATG CTC CTT CAG GTC CAC Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His 770 775 780	2472
45	GAC GAG CTG GTC CTC GAG GCC CCA AAA GAG AGG GCG GAG GCC GTG GCC Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala 785 790 795 800	2520
50	CGG CTG GCC AAG GAA GTC ATG GAG GGG GTG TAT CCC CTG GCC GTG CCC Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro 805 810 815	2568
55	CTG GAG GTG GAG GTG GGG ATA GGG GAG GAC TGG CTC TCC GCC AAG GAG Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu 820 825 830	2616
	TGATACCACC	2626

60 (2) INFORMATION FOR SEQ ID NO::6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 832 amino acids

49

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO::6:

5 Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu
1 5 10 15

10 Val Asp Gly His His Leu Ala Tyr Cys Thr Phe His Ala Leu Lys Gly
20 25 30

15 Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala
35 40 45

Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val
50 55 60

20 Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly
65 70 75 80

Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu
85 90 95

25 Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu
100 105 110

30 Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys
115 120 125

Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp
130 135 140

35 Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly
145 150 155 160

Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro
165 170 175

40 Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn
180 185 190

45 Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu
195 200 205

Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu
210 215 220

50 Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys
225 230 235 240

Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val
245 250 255

55 Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Ile
260 265 270

60 Leu Glu Arg Leu Glu Phe Gly Ser Pro Leu His Glu Phe Gly Leu Leu
275 280 285

Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly
290 295 300

50

Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp
 305 310 315 320
 5 Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro
 325 330 335
 Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu
 340 345 350
 10 Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro
 355 360 365
 Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn
 370 375 380
 15 Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu
 385 390 395 400
 Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu
 405 410 415
 20 Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu
 420 425 430
 25 Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly
 435 440 445
 Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala
 450 455 460
 30 Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His
 465 470 475 480
 Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp
 485 490 495
 35 Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg
 500 505 510
 40 Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile
 515 520 525
 Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr
 530 535 540
 45 Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu
 545 550 555 560
 His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser
 565 570 575
 50 Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln
 580 585 590
 Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala
 595 600 605
 55 Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly
 610 615 620
 60 Asp Leu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr
 625 630 635 640

51

Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro
 645 650 655
 5 Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly
 660 665 670
 Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu
 675 680 685
 10 Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg
 690 695 700
 Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val
 705 710 715 720
 15 Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg
 725 730 735
 Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro
 740 745 750
 Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu
 755 760 765
 25 Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His
 770 775 780
 Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala
 785 790 795 800
 30 Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro
 805 810 815
 Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu
 820 825 830

(2) INFORMATION FOR SEQ ID NO::7:

- 40 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 2626 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 45 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

50 (iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Thermus aquaticus*

55 (ix) FEATURE:

(A) NAME/KEY: mutation

(B) LOCATION: replace(89, "g")

60 (D) OTHER INFORMATION: /note= "This mutation results in a
 nucleotide alteration at position 89 of the native Taq DNA
 polymerase nucleotide sequence of C to G."

(ix) FEATURE:

52

(A) NAME/KEY: mutation
 (B) LOCATION: replace(934, "a")
 (D) OTHER INFORMATION: /note= "This mutation results in a
 nucleotide alteration at position 934 of the native Taq
 DNA polymerase nucleotide sequence of T.to A. This
 results in an amino acid change of Phe to Ile."

(ix) FEATURE:
 (A) NAME/KEY: mutation
 (B) LOCATION: replace(962, "c")
 (D) OTHER INFORMATION: /note= "This mutation results in a
 nucleotide alteration at position 962 of the native Taq
 DNA polymerase nucleotide sequence of T to C. This
 results in an amino acid change of Leu to Pro."

(ix) FEATURE:
 (A) NAME/KEY: mutation
 (B) LOCATION: replace(2535, "a")
 (D) OTHER INFORMATION: /note= "This mutation results in a
 nucleotide alteration at position 2535 of the native Taq
 DNA polymerase nucleotide sequence of G to A. This
 mutation is conservative."

(ix) FEATURE:
 (A) NAME/KEY: mutation
 (B) LOCATION: replace(341, "a")
 (D) OTHER INFORMATION: /note= "This mutation results in a
 nucleotide alteration at position 341 of the native Taq
 DNA polymerase nucleotide sequence of G to A. This
 mutation results in an amino acid change of Arg to His."

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 121..2619

(ix) FEATURE:
 (A) NAME/KEY: mat_peptide
 (B) LOCATION: 121..2616

(ix) FEATURE:
 (A) NAME/KEY: -
 (B) LOCATION: 1..2619
 (D) OTHER INFORMATION: /note= "pTarf5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO::7:

	AAGCTCAGAT CTACCTGCCT GAGGGCGTCC GGTTCAGCT GGCCCTTCCC GAGGGGGAGA	60
50	GGGAGGCGTT TCTAAAAGCC CTCAGGAGG CTACCGGGG GCGGGTGGTG GAAGGGTAAC	120
	ATG AGG GGG ATG CTG CCC CTC TTT GAG CCC AAG GGC CGG GTC CTC CTG	168
	Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu	
	1 5 10 15	
55	GTG GAC GGC CAC CAC CTG GCC TAC CGC ACC TTC CAC CC CTG AAG GGC	216
	Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly	
	20 25 30	
60	CTC ACC ACC AGC CGG GGG GAG CCG GTG CAG GCG GTC TAC GGC TTC GCC	264
	Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala	
	35 40 45	

53

	AAG AGC CTC CTC AAG GCC CTC AAG GAG GAC GGG GAC GCG GTG ATC GTG Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val	312
	50 55 60	
5	GTC TTT GAC GCC AAG GCC CCC TCC TTC CAC CAC GAG GCC TAC GGG GGG Val Phe Asp Ala Lys Ala Pro Ser Phe His His Glu Ala Tyr Gly Gly	360
	65 70 75 80	
10	TAC AAG GCG GGC CGG GCC CCC ACG CCG GAG GAC TTT CCC CGG CAA CTC Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu	408
	85 90 95	
15	GCC CTC ATC AAG GAG CTG GTG GAC CTC CTG GGG CTG GCG CGC CTC GAG Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu	456
	100 105 110	
20	GTC CCG GGC TAC GAG GCG GAC GAC GTC CTG GCC AGC CTG GCC AAG AAG Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys	504
	115 120 125	
25	GCG GAA AAG GAG GGC TAC GAG GTC CGC ATC CTC ACC GCC GAC AAA GAC Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp	552
	130 135 140	
30	CTT TAC CAG CTC CTT TCC GAC CGC ATC CAC GTC CTC CAC CCC GAG GGG Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly	600
	145 150 155 160	
35	TAC CTC ATC ACC CCG GCC TGG CTT TGG GAA AAG TAC GGC CTG AGG CCC Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro	648
	165 170 175	
40	GAC CAG TGG GCC GAC TAC CGG GCC CTG ACC GGG GAC GAG TCC GAC AAC Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn	696
	180 185 190	
45	CTT CCC GGG GTC AAG GGC ATC GGG GAG AAG ACG GCG AGG AAG CTT CTG Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu	744
	195 200 205	
50	GAG GAG TGG GGG AGC CTG GAA GCC CTC CTC AAG AAC CTG GAC CGG CTG Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu	792
	210 215 220	
55	AAG CCC GCC ATC CGG GAG AAG ATC CTG GCC CAC ATG GAC GAT CTG AAG Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys	840
	225 230 235 240	
60	CTC TCC TGG GAC CTG GCC AAG GTG CGC ACC GAC CTG CCC CTG GAG GTG Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val	888
	245 250 255	
65	GAC TTC GCC AAA AGG CGG GAG CCC GAC CGG GAG AGG CTT AGG GCC ATT Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Ile	936
	260 265 270	
70	CTG GAG AGG CTT GAG TTT GGC AGC CCC CTC CAC GAG TTC GGC CTT CTG Leu Glu Arg Leu Glu Phe Gly Ser Pro Leu His Glu Phe Gly Leu Leu	984
	275 280 285	
75	GAA AGC CCC AAG GCC CTG GAG GAG GCC CCC TGG CCC CCG CCG GAA GGG Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Glu Gly	1032
	290 295 300	

54

	GCC TTC GTG GGC TTT GTG CTT TCC CGC AAG GAG CCC ATG TGG GCC GAT	1080
	Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp	
	305 310 315 320	
5	CTT CTG GCC CTG GCC GCC GCC AGG GGG GGC CGG GTC CAC CGG GGC CCC	1128
	Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro	
	325 330 335	
10	GAG CCT TAT AAA GCC CTC AGG GAC CTG AAG GAG GCG CGG GGG CTT CTC	1176
	Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu	
	340 345 350	
15	GCC AAA GAC CTG AGC GTT CTG GCC CTG AGG GAA GGC CTT GGC CTC CCG	1224
	Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro	
	355 360 365	
20	CCC GGC GAC GAC CCC ATG CTC CTC GCC TAC CTC CTG GAC CCT TCC AAC	1272
	Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn	
	370 375 380	
25	ACC ACC CCC GAG GGG GTG GCC CGG CGC TAC GGC GGG GAG TGG ACG GAG	1320
	Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu	
	385 390 395 400	
30	GAG GCG GGG GAG CGG GCC GCC CTT TCC GAG AGG CTC TTC GCC AAC CTG	1368
	Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu	
	405 410 415	
35	TGG GGG AGG CTT GAG GGG GAG GAG AGG CTC CTT TGG CTT TAC CGG GAG	1416
	Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu	
	420 425 430	
40	GTG GAG AGG CCC CTT TCC GCT GTC CTG GCC CAC ATG GAG GCC ACG GGG	1464
	Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly	
	435 440 445	
45	GTG CGC CTG GAC GTG GCC TAT CTC AGG GCC TTG TCC CTG GAG GTG GCC	1512
	Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala	
	450 455 460	
50	GAG GAG ATC GCC CGC CTC GAG GCC GAG GTC TTC CGC CTG GCC GGC CAC	1560
	Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His	
	465 470 475 480	
55	CCC TTC AAC CTC AAC TCC CGG GAC CAG CTG GAA AGG GTC CTC TTT GAC	1608
	Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp	
	485 490 495	
60	GAG CTA GGG CTT CCC GCC ATC GGC AAG ACG GAG AAG ACC GGC AAG CGC	1656
	Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg	
	500 505 510	
65	TCC ACC AGC GCC GCC GTC CTG GAG GCC CTC CGC GAG GCC CAC CCC ATC	1704
	Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile	
	515 520 525	
70	GTG GAG AAG ATC CTG CAG TAC CGG GAG CTC ACC AAG CTG AAG AGC ACC	1752
	Val Leu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr	
	530 535 540	
75	TAC ATT GAC CCC TTG CCG GAC CTC ATC CAC CCC AGG ACG GGC CGC CTC	1800
	Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu	
	545 550 555 560	

55

	CAC ACC CGC TTC AAC CAG ACG GCC ACG GCC ACG GGC AGG CTA AGT AGC His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser	1848
	565 570 575	
5	TCC GAT CCC AAC CTC CAG AAC ATC CCC GTC CGC ACC CCG CTT GGG CAG Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln	1896
	580 585 590	
10	AGG ATC CGC CGG GCC TTC ATC GCC GAG GAG GGG TGG CTA TTG GTG GCC Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala	1944
	595 600 605	
15	CTG GAC TAT AGC CAG ATA GAG CTC AGG GTG CTG GCC CAC CTC TCC GGC Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly	1992
	610 615 620	
20	GAC GAG AAC CTG ATC CGG GTC TTC CAG GAG GGG CGG GAC ATC CAC ACG Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr	2040
	625 630 635 640	
25	GAG ACC GCC AGC TGG ATG TTC GGC GTC CCC CGG GAG GCC GTG GAC CCC Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro	2088
	645 650 655	
30	CTG ATG CGC CGG GCG GCC AAG ACC ATC AAC TTC GGG GTC CTC TAC GGC Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly	2136
	660 665 670	
35	ATG TCG GCC CAC CGC CTC TCC CAG GAG CTA GCC ATC CCT TAC GAG GAG Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu	2184
	675 680 685	
40	GCC CAG GCC TTC ATT GAG CGC TAC TTT CAG AGC TTC CCC AAG GTG CGG Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg	2232
	690 695 700	
45	GCC TGG ATT GAG AAG ACC CTG GAG GAG GGC AGG AGG CGG GGG TAC GTG Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val	2280
	705 710 715 720	
50	GAG ACC CTC TTC GGC CGC CGC CGC TAC GTG CCA GAC CTA GAG GCC CGG Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg	2328
	725 730 735	
55	GTG AAG AGC GTG CGG GAG GCG GCC GAG CGC ATG GCC TTC AAC ATG CCC Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro	2376
	740 745 750	
60	GTC CAG GGC ACC GCC GCC GAC CTC ATG AAG CTG GCT ATG GTG AAG CTC Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu	2424
	755 760 765	
65	TTC CCC AGG CTG GAG GAA ATG GGG GCC AGG ATG CTC CTT CAG GTC CAC Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His	2472
	770 775 780	
70	GAC GAG CTG GTC CTC GAG GCC CCA AAA GAG AGG GCG GAG GCC GTG GCC Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala	2520
	785 790 795 800	
75	CGG CTG GCC AAG GAA GTC ATG GAG GGG GTG TAT CCC CTG GCC GTG CCC Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro	2568
	805 810 815	

56

CTG GAG GTG GAG GTG GGG ATA GGG GAG GAC TGG CTC TCC GCC AAG GAG 2616
Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu
820 825 830

5 TGATACCACC . 2626

(2) INFORMATION FOR SEQ ID NO::8:

10 (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 832 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO::8:

20 Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu
1 5 10 15

Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly
20 25 30

25 Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala
 35 40 45

Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val
50 55 60

Val Phe Asp Ala Lys Ala Pro Ser Phe His His Glu Ala Tyr Gly Gly
65 70 75 80

35 Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu
85 90 95

Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu
100 105 110

40 Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys
115 120 125

Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp
130 135 140

Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly
145 150 155 160

50 Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro
165 170 175

Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn
180 185 190

55 Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu
 195 200 205

Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu
210 215 220

60

Lys	Pro	Ala	Ile	Arg	Glu	Lys	Ile	Leu	Ala	His	Met	Asp	Asp	Leu	Lys
225					230					235					240

57

Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val
 245 250 255
 5 Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Ile
 260 265 270
 Leu Glu Arg Leu Glu Phe Gly Ser Pro Leu His Glu Phe Gly Leu Leu
 275 280 285
 10 Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly
 290 295 300
 Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp
 305 310 315 320
 15 Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro
 325 330 335
 20 Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu
 340 345 350
 Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro
 355 360 365
 25 Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn
 370 375 380
 30 Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu
 385 390 395 400
 Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu
 405 410 415
 35 Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu
 420 425 430
 Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly
 435 440 445
 40 Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala
 450 455 460
 Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His
 465 470 475 480
 45 Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp
 485 490 495
 50 Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg
 500 505 510
 Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile
 515 520 525
 55 Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr
 530 535 540
 Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu
 545 550 555 560
 60 His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser
 565 570 575

58

Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln
 580 585 590
 5 Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala
 595 600 605
 Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly
 610 615 620
 10 Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr
 625 630 635 640
 Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro
 645 650 655
 15 Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly
 660 665 670
 Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu
 675 680 685
 20 Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg
 690 695 700
 Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val
 705 710 715 720
 Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg
 725 730 735
 30 Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro
 740 745 750
 Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu
 755 760 765
 35 Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His
 770 775 780
 Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala
 785 790 795 800
 Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro
 805 810 815
 45 Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu
 820 825 830

50 (2) INFORMATION FOR SEQ ID NO.:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2626 base pairs
 (B) TYPE: nucleic acid
 55 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

60 (iii) HYPOTHETICAL: NO

(ix) FEATURE:

- (A) NAME/KEY: mutation

59

(B) LOCATION: replace(89, "g")
 (D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at position 89 of the native Taq DNA polymerase nucleotide sequence of C to G."

(ix) FEATURE:

(A) NAME/KEY: mutation
 (B) LOCATION: replace(934, "a")
 (D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at position 934 of the native Taq DNA polymerase nucleotide sequence of T to A. This results in an amino acid change of Phe to Ile."

(ix) FEATURE:

(A) NAME/KEY: mutation
 (B) LOCATION: replace(962, "c")
 (D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at position 962 of the native Taq DNA polymerase nucleotide sequence of T to C. This results in an amino acid change of Leu to Pro."

(ix) FEATURE:

(A) NAME/KEY: mutation
 (B) LOCATION: replace(2535, "a")
 (D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at position 2535 of the native Taq DNA polymerase nucleotide sequence of G to A. This mutation is conservative."

(ix) FEATURE:

(A) NAME/KEY: mutation
 (B) LOCATION: replace(337, "a")
 (D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at position 337 of the native Taq DNA polymerase nucleotide sequence of T to C. This change results in an amino acid change of Phe to Leu."

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 121..2619

(ix) FEATURE:

(A) NAME/KEY: mat_peptide
 (B) LOCATION: 121..2616

(ix) FEATURE:

(A) NAME/KEY: -
 (B) LOCATION: 1..2619
 (D) OTHER INFORMATION: /note= "pTarf2"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO::9:

AAGCTCAGAT CTACCTGCCT GAGGGCGTCC GGTTCAGCT GGCCCTTCCC GAGGGGGAGA	60
GGGAGGCGTT TCTAAAAGCC CTCAGGAGG CTACCGGGG GCGGGTGGTG GAAGGGTAAC	120
ATG AGG GGG ATG CTG CCC CTC TTT GAG CCC AAG GGC CGG GTC CTC CTG	168
Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu	
1 5 10 15	
GTG GAC GGC CAC CAC CTG GCC TAC CGC ACC TTC CAC GCC CTG AAG GGC	216

60

	Val	Asp	Gly	His	His	Leu	Ala	Tyr	Arg	Thr	Phe	His	Ala	Leu	Lys	Gly	
				20					25					30			
5	CTC	ACC	ACC	AGC	CGG	GGG	GAG	CCG	GTG	CAG	GCG	GTC	TAC	GGC	TTC	GCC	264
	Leu	Thr	Thr	Ser	Arg	Gly	Glu	Pro	Val	Gln	Ala	Val	Tyr	Gly	Phe	Ala	
			35					40					45				
10	AAG	AGC	CTC	CTC	AAG	GCC	CTC	AAG	GAG	GAC	GGG	GAC	GCG	GTG	ATC	GTG	312
	Lys	Ser	Leu	Leu	Lys	Ala	Leu	Lys	Glu	Asp	Gly	Asp	Ala	Val	Ile	Val	
			50				55					60					
15	GTC	TTT	GAC	GCC	AAG	GCC	CCC	TCC	CTC	CGC	CAC	GAG	GCC	TAC	GGG	GGG	360
	Val	Phe	Asp	Ala	Lys	Ala	Pro	Ser	Leu	Arg	His	Glu	Ala	Tyr	Gly	Gly	
	65				70					75					80		
20	TAC	AAG	GCG	GGC	CGG	GCC	CCC	ACG	CCG	GAG	GAC	TTT	CCC	CGG	CAA	CTC	408
	Tyr	Lys	Ala	Gly	Arg	Ala	Pro	Thr	Pro	Glu	Asp	Phe	Pro	Arg	Gln	Leu	
				85					90					95			
25	GCC	CTC	ATC	AAG	GAG	CTG	GTG	GAC	CTC	CTG	GGG	CTG	GCG	CGC	CTC	GAG	456
	Ala	Leu	Ile	Lys	Glu	Leu	Val	Asp	Leu	Leu	Gly	Leu	Ala	Arg	Leu	Glu	
				100				105						110			
30	GTC	CCG	GGC	TAC	GAG	GCG	GAC	GAC	GTC	CTG	GCC	AGC	CTG	GCC	AAG	AAG	504
	Val	Pro	Gly	Tyr	Glu	Ala	Asp	Asp	Val	Leu	Ala	Ser	Leu	Ala	Lys	Lys	
			115				120						125				
35	GCG	GAA	AAG	GAG	GGC	TAC	GAG	GTC	CGC	ATC	CTC	ACC	GCC	GAC	AAA	GAC	552
	Ala	Glu	Lys	Glu	Gly	Tyr	Glu	Val	Arg	Ile	Leu	Thr	Ala	Asp	Lys	Asp	
	130				135					140							
40	CTT	TAC	CAG	CTC	CTT	TCC	GAC	CGC	ATC	CAC	GTC	CTC	CAC	CCC	GAG	GGG	600
	Leu	Tyr	Gln	Leu	Leu	Ser	Asp	Arg	Ile	His	Val	Leu	His	Pro	Glu	Gly	
	145				150					155					160		
45	TAC	CTC	ATC	ACC	CCG	GCC	TGG	CTT	TGG	GAA	AAG	TAC	GGC	CTG	AGG	CCC	648
	Tyr	Leu	Ile	Thr	Pro	Ala	Trp	Leu	Trp	Glu	Lys	Tyr	Gly	Leu	Arg	Pro	
				165				170						175			
50	GAC	CAG	TGG	GCC	GAC	TAC	CGG	GCC	CTG	ACC	GGG	GAC	GAG	TCC	GAC	AAC	696
	Asp	Gln	Trp	Ala	Asp	Tyr	Arg	Ala	Leu	Thr	Gly	Asp	Glu	Ser	Asp	Asn	
				180				185						190			
55	CTT	CCC	GGG	GTC	AAG	GGC	ATC	GGG	GAG	AAG	ACG	GCG	AGG	AAG	CTT	CTG	744
	Leu	Pro	Gly	Val	Lys	Gly	Ile	Gly	Glu	Lys	Thr	Ala	Arg	Lys	Leu	Leu	
			195				200						205				
60	GAG	GAG	TGG	GGG	AGC	CTG	GAA	GCC	CTC	CTC	AAG	AAC	CTG	GAC	CGG	CTG	792
	Glu	Glu	Trp	Gly	Ser	Leu	Glu	Ala	Leu	Leu	Lys	Asn	Leu	Asp	Arg	Leu	
			210			215						220					
65	AAG	CCC	GCC	ATC	CGG	GAG	AAG	ATC	CTG	GCC	CAC	ATG	GAC	GAT	CTG	AAG	840
	Lys	Pro	Ala	Ile	Arg	Glu	Lys	Ile	Leu	Ala	His	Met	Asp	Asp	Leu	Lys	
	225				230					235					240		
70	CTC	TCC	TGG	GAC	CTG	GCC	AAG	GTG	CGC	ACC	GAC	CTG	CCC	CTG	GAG	GTG	888
	Leu	Ser	Trp	Asp	Leu	Ala	Lys	Val	Arg	Thr	Asp	Leu	Pro	Leu	Glu	Val	
				245					250					255			
75	GAC	TTC	GCC	AAA	AGG	CGG	GAG	CCC	GAC	CGG	GAG	AGG	CTT	AGG	GCC	ATT	936
	Asp	Phe	Ala	Lys	Arg	Arg	Glu	Pro	Asp	Arg	Glu	Arg	Leu	Arg	Ala	Ile	
				260				265						270			

61

	CTG GAG AGG CTT GAG TTT GGC AGC CCC CTC CAC GAG TTC GGC CTT CTG Leu Glu Arg Leu Glu Phe Gly Ser Pro Leu His Glu Phe Gly Leu Leu 275 280 285	984
5	GAA AGC CCC AAG GCC CTG GAG GAG GCC CCC TGG CCC CCG CCG GAA GGG Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly 290 295 300	1032
10	GCC TTC GTG GGC TTT GTG CTT TCC CGC AAG GAG CCC ATG TGG GCC GAT Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp 305 310 315 320	1080
15	CTT CTG GCC CTG GCC GCC GCC AGG GGG GGC CGG GTC CAC CGG GCC CCC Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro 325 330 335	1128
20	GAG CCT TAT AAA GCC CTC AGG GAC CTG AAG GAG GCG CGG GGG CTT CTC Glu Pro Tyr Lys Ala Leu Arg Asp Lys Glu Ala Arg Gly Leu Leu 340 345 350	1176
25	GCC AAA GAC CTG AGC GTT CTG GCC CTG AGG GAA GGC CTT GGC CTC CCG Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro 355 360 365	1224
30	CCC GGC GAC GAC CCC ATG CTC CTC GCC TAC CTC CTG GAC CCT TCC AAC Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn 370 375 380	1272
35	ACC ACC CCC GAG GGG GTG GCC CGG CGC TAC GGC GGG GAG TGG ACG GAG Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu 385 390 395 400	1320
40	GAG GCG GGG GAG CGG GCC GCC CTT TCC GAG AGG CTC TTC GCC AAC CTG Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu 405 410 415	1368
45	TGG GGG AGG CTT GAG GGG GAG GAG AGG CTC CTT TGG CTT TAC CGG GAG Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu 420 425 430	1416
50	GTG GAG AGG CCC CTT TCC GCT GTC CTG GCC CAC ATG GAG GCC ACG GGG Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly 435 440 445	1464
55	GTG CGC CTG GAC GTG GCC TAT CTC AGG GCC TTG TCC CTG GAG GTG GCC Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala 450 455 460	1512
60	GAG GAG ATC GCC CGC CTC GAG GCC GAG GTC TTC CGC CTG GCC GGC CAC Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His 465 470 475 480	1560
	CCC TTC AAC CTC AAC TCC CGG GAC CAG CTG GAA AGG GTC CTC TTT GAC Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp 485 490 495	1608
	GAG CTA GGG CTT CCC GCC ATC GGC AAG ACG GAG AAG ACC GGC AAG CGC Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg 500 505 510	1656
	TCC ACC AGC GCC GCC GTC CTG GAG GCC CTC CGC GAG GCC CAC CCC ATC Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile 515 520 525	1704

62

	GTG GAG AAG ATC CTG CAG TAC CGG GAG CTC ACC AAG CTG AAG AGC ACC Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr 530 535 540	1752
5	TAC ATT GAC CCC TTG CCG GAC CTC ATC CAC CCC AGG ACG GGC CGC CTC Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu 545 550 555 560	1800
10	CAC ACC CGC TTC AAC CAG ACG GCC ACG GCC ACG GGC AGG CTA AGT AGC His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser 565 570 575	1848
15	TCC GAT CCC AAC CTC CAG AAC ATC CCC GTC CGC ACC CCG CTT GGG CAG Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln 580 585 590	1896
20	AGG ATC CGC CGG GCC TTC ATC GCC GAG GAG GGG TGG CTA TTG GTG GCC Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala 595 600 605	1944
25	CTG GAC TAT AGC CAG ATA GAG CTC AGG GTG CTG GCC CAC CTC TCC GGC Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly 610 615 620	1992
30	GAC GAG AAC CTG ATC CGG GTC TTC CAG GAG GGG CGG GAC ATC CAC ACG Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr 625 630 635 640	2040
35	GAG ACC GCC AGC TGG ATG TTC GGC GTC CCC CGG GAG GCC GTG GAC CCC Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro 645 650 655	2088
40	CTG ATG CGC CGG GCG GCC AAG ACC ATC AAC TTC GGG GTC CTC TAC GGC Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly 660 665 670	2136
45	ATG TCG GCC CAC CGC CTC TCC CAG GAG CTA GCC ATC CCT TAC GAG GAG Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu 675 680 685	2184
50	GCC CAG GCC TTC ATT GAG CGC TAC TTT CAG AGC CCC AAG GTG CGG Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg 690 695 700	2232
55	GCC TGG ATT GAG AAG ACC CTG GAG GAG GGC AGG AGG CGG GGG TAC GTG Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val 705 710 715 720	2280
60	GAG ACC CTC TTC GGC CGC CGC CGC TAC GTG CCA GAC CTA GAG GCC CGG Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg 725 730 735	2328
65	GTG AAG AGC GTG CGG GAG GCG GCC GAG CGC ATG GCC TTC AAC ATG CCC Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro 740 745 750	2376
70	GTC CA GGC ACC GCC GCC GAC CTC ATG AAG CTG GCT ATG GTG AAG CTC Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu 755 760 765	2424
75	TTC CCC AGG CTG GAG GAA ATG GGG GCC AGG ATG CTC CTT CAG GTC CAC Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His 770 775 780	2472

63

	GAC GAG CTG GTC CTC GAG GCC CCA AAA GAG AGG GCG GAG GCC GTG GCC	2520
	Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala	
	785 790 795 800	
5	CGG CTG GCC AAG GAA GTC ATG GAG GGG GTG TAT CCC CTG GCC GTG CCC	2568
	Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro	
	805 810 815	
10	CTG GAG GTG GAG GTG GGG ATA GGG GAG GAC TGG CTC TCC GCC AAG GAG	2616
	Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu	
	820 825 830	
	TGATACCACC	2626
15	(2) INFORMATION FOR SEQ ID NO::10:	
	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 832 amino acids	
20	(B) TYPE: amino acid	
	(D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: protein	
25	(xi) SEQUENCE DESCRIPTION: SEQ ID NO::10:	
	Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu	
	1 5 10 15	
30	Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly	
	20 25 30	
	Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala	
35	35 40 45	
	Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val	
	50 55 60	
40	Val Phe Asp Ala Lys Ala Pro Ser Leu Arg His Glu Ala Tyr Gly Gly	
	65 70 75 80	
	Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu	
	85 90 95	
45	Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu	
	100 105 110	
	Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys	
50	115 120 125	
	Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp	
	130 135 140	
55	Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly	
	145 150 155 160	
	Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro	
	165 170 175	
60	Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn	
	180 185 190	

64

Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu
 195 200 205
 5 Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu
 210 215 220
 Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys
 225 230 235 240
 10 Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val
 245 250 255
 Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Ile
 260 265 270
 15 Leu Glu Arg Leu Glu Phe Gly Ser Pro Leu His Glu Phe Gly Leu Leu
 275 280 285
 Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly
 290 295 300
 20 Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp
 305 310 315 320
 25 Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro
 325 330 335
 Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu
 340 345 350
 30 Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro
 355 360 365
 35 Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn
 370 375 380
 Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu
 385 390 395 400
 40 Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu
 405 410 415
 Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu
 420 425 430
 45 Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly
 435 440 445
 Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala
 450 455 460
 Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His
 465 470 475 480
 55 Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp
 485 490 495
 Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg
 500 505 510
 60 Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile
 515 520 525

65

Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr
530 535 540

5 Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu
545 550 555 560

His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser
565 570 575

10 Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln
580 585 590

Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala
595 600 605

15 Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly
610 615 620

20 Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr
625 630 635 640

Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro
645 650 655

25 Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly
660 665 670

Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu
675 680 685

30 Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg
690 695 700

Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val
705 710 715 720

35 Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg
725 730 735

40 Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro
740 745 750

Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu
755 760 765

45 Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His
770 775 780

Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala
785 790 795 800

Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro
805 810 815

55 Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu
820 825 830

(2) INFORMATION FOR SEQ ID NO.:11:

60

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 base pairs
 (B) TYPE: nucleic acid

66

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION: 1..18
(D) OTHER INFORMATION: /note= "PCR reverse primer used for PUC18"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO::11:
CAGGAAACAG CTATGACC 18

(2) INFORMATION FOR SEQ ID NO::12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION: 11..15
(D) OTHER INFORMATION: /note= "PCR sequencing primer 628A used for pUC18"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO::12:
CCCAAAGCCA GGCOG 15

(2) INFORMATION FOR SEQ ID NO::13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION: 1..15
(D) OTHER INFORMATION: /note= "Sequencing primer 1155A"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO::13:

67

CAGGTCCCTG AGGGC

15

(2) INFORMATION FOR SEQ ID NO::14:

- 5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
10 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- 15 (ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION: 1..46
20 (D) OTHER INFORMATION: /note= "pUC18 - pLSM5 5' junction"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO::14:

AATTTACAC AGGAAACAGC TATGACCATG ATTACGAATT CTAAAA

46

(2) INFORMATION FOR SEQ ID NO::15:

- 30 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 63 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- 35 (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- 40 (ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION: 1..63
(D) OTHER INFORMATION: /note= "pUC18 - pLSM5 3' sequence
junction"

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO::15:

CAAGGAGTGA GATTCTCTAG AGTCGACCTG CAGGCATGCA AGCTTGGCAC TGGCCGTCGT
50 TTT

60

63

What is claimed is:

1. A modified *Taq* DNA polymerase gene essentially comprising the native *Taq* DNA polymerase gene having an altered nucleotide at positions 89, 934, 962, and 2535.
- 5 2. A modified *Taq* DNA polymerase gene according to claim 1 wherein the altered nucleotide at position 89 is G, the altered nucleotide at position 934 is A, the altered nucleotide at position 962 is C, and the altered amino acid at position 2525 is A.
- 10 3. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 1.
4. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 2.
5. A modified *Taq* DNA polymerase essentially comprising the native *Taq* DNA polymerase with an altered amino acid at positions 272 and 281.
- 15 6. A modified *Taq* DNA polymerase according to claim 5 wherein the altered amino acid at position 272 is Ile and the altered amino acid at position 281 is Pro.
7. A modified *Taq* DNA polymerase gene essentially comprising the native *Taq* DNA polymerase gene having an altered nucleotide at positions 193 and 504.
- 20 8. A modified *Taq* polymerase gene according to claim 7 wherein the altered nucleotide at position 193 is T and the altered nucleotide at position 504 is A.

9. A modified *Taq* DNA polymerase gene essentially comprising the native *Taq* DNA polymerase gene having an altered nucleotide at positions 89, 193, 504, 934, 962, and 2535.

10. A modified *Taq* polymerase gene according to claim 9 wherein the
5 altered nucleotide at position 89 is G, the altered amino acid at position 193 is T, the altered amino acid at position 504 is A, the altered nucleotide at position 934 is A, the altered nucleotide at position 962 is C, and the altered amino acid at position 2525 is A.

10 11. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 7.

12. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 8.

13. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 9.

15 14. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 10.

15. A modified *Taq* DNA polymerase essentially comprising the native *Taq* DNA polymerase with an altered amino acid at position 25.

20 16. A modified *Taq* DNA polymerase according to claim 15 wherein the altered amino acid at position 25 is Cys.

17. A modified *Taq* DNA polymerase essentially comprising the native *Taq* DNA polymerase with an altered amino acid at positions 25, 272, and 281

18. A modified *Taq* DNA polymerase according to claim 17 wherein the altered amino acid at position 25 is Cys, the altered amino acid at position 272 is Ile, and the altered amino acid at position 281 is Pro.

5 19. A modified *Taq* DNA polymerase gene essentially comprising the native *Taq* DNA polymerase gene having an altered nucleotide at position 341.

20. A modified *Taq* polymerase gene according to claim 19 wherein the altered nucleotide at position 193 is T and the altered nucleotide at position 504 is A.

10 21. A modified *Taq* DNA polymerase gene essentially comprising the native *Taq* DNA polymerase gene having an altered nucleotide at positions 89, 341, 934, 962, and 2535.

22. A modified *Taq* polymerase gene according to claim 21 wherein the altered nucleotide at position 89 is G, the altered amino acid at position 341 is A, the altered nucleotide at position 934 is A, the altered nucleotide at position 962 is C, and the altered amino acid at position 2525 is A

15

23. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 19.

24. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 20.

20 25. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 21.

26. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 22.

27. A modified *Taq* DNA polymerase essentially comprising the native *Taq* DNA polymerase with an altered amino acid at position 74.

28. A modified *Taq* DNA polymerase according to claim 27 wherein the altered amino acid at position 74 is His.

5 29. A modified *Taq* DNA polymerase essentially comprising the native *Taq* DNA polymerase with an altered amino acid at positions 74, 272, and 281

30. A modified *Taq* DNA polymerase according to claim 29 wherein the altered amino acid at position 74 is His, the altered amino acid at position 272 is Ile, and the altered amino acid at position 281 is Pro.

10 31. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 5.

32. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 6.

15 33. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 15.

34. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 16.

35. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 17.

20 36. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 18.

37. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 27.

38. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 38.

39. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 29.

5 40. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 30.

41. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 5.

10 42. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 6.

43. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 15.

44. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 16.

15 45. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 17.

46. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 18.

20 47. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 27.

48. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 28.

49. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 29.

50. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 30.

5 51. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 5.

52. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 6.

10 53. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 15.

54. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 16.

55. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 17.

15 56. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 18

57. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 27.

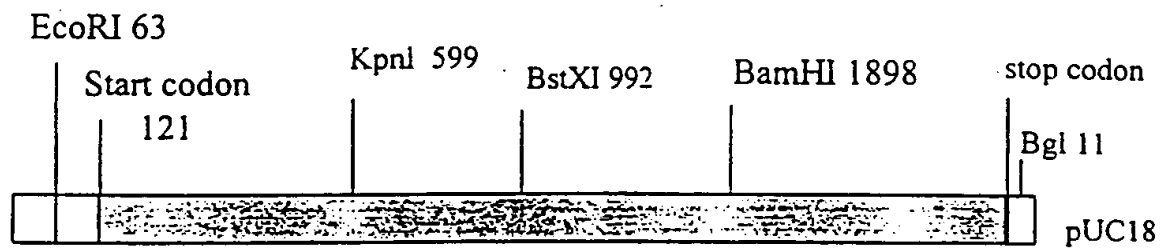
20 58. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 28.

59. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 29.

60. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 30.

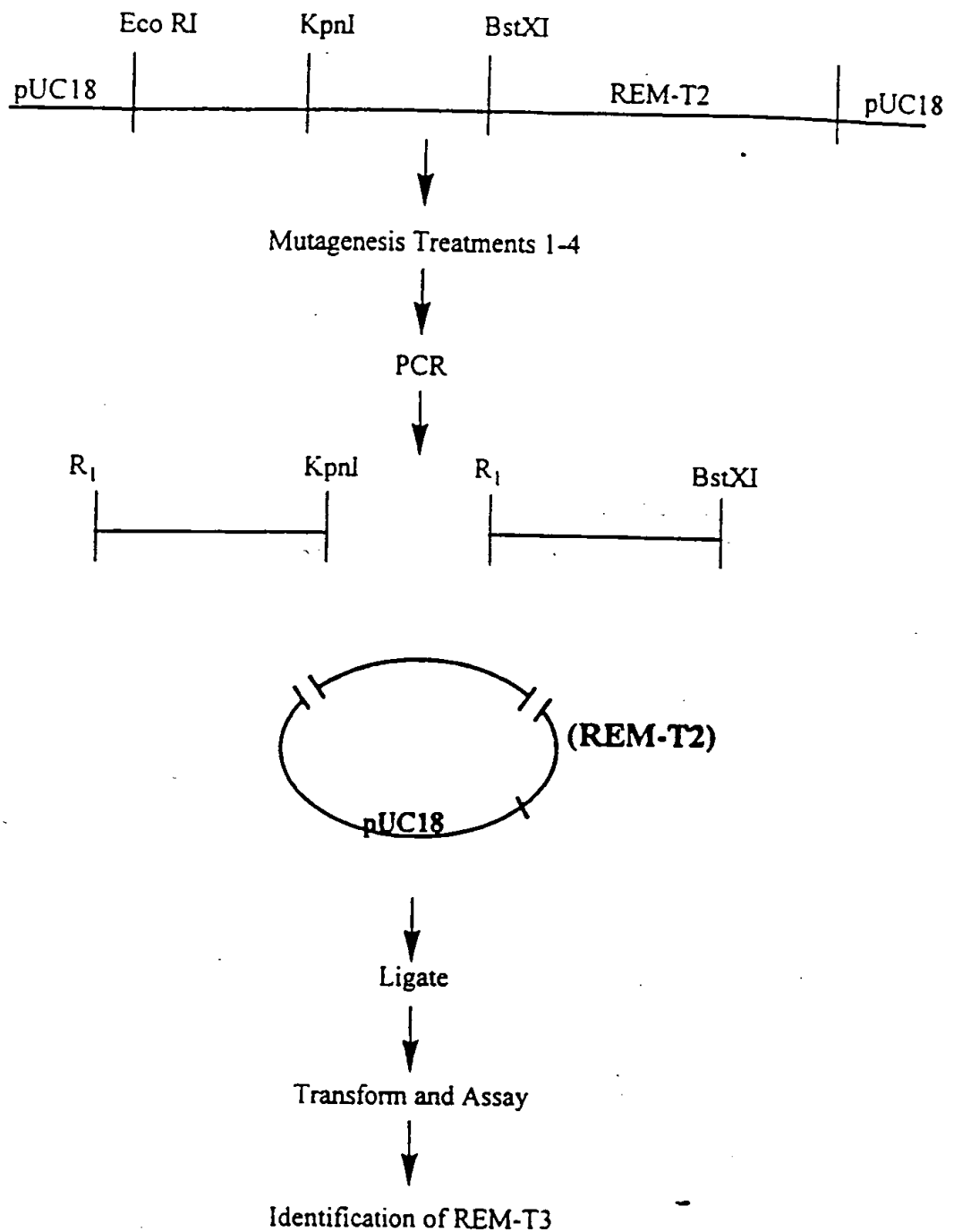
1 / 8

FIG. 1



Restriction map of gene for Taq DNA polymerase

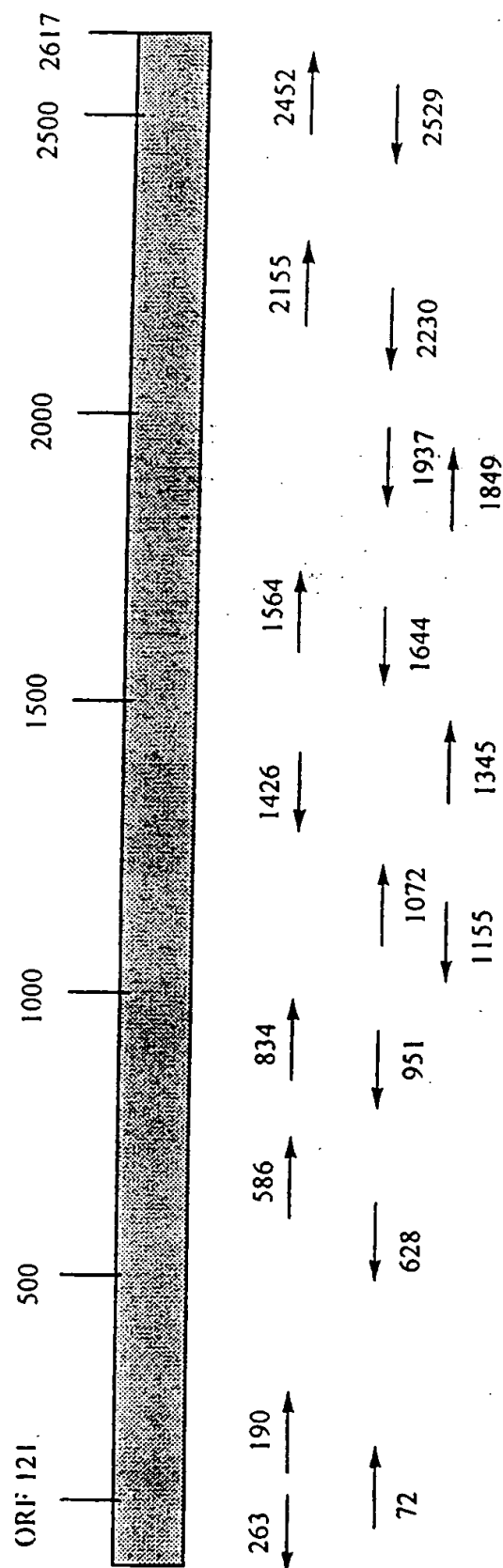
2 / 8

FIG. 2**Scheme for Zone Mutagenesis**

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FIG. 3

Sequencing Primers for pLSM5



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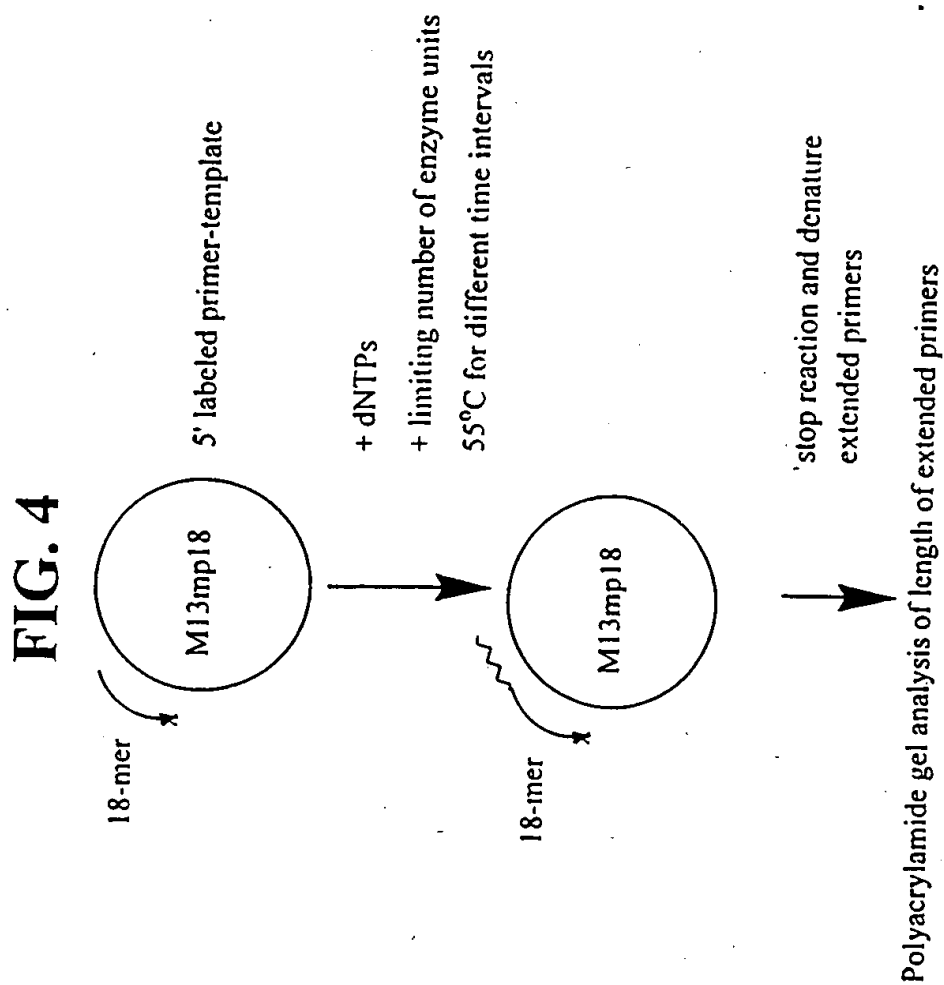
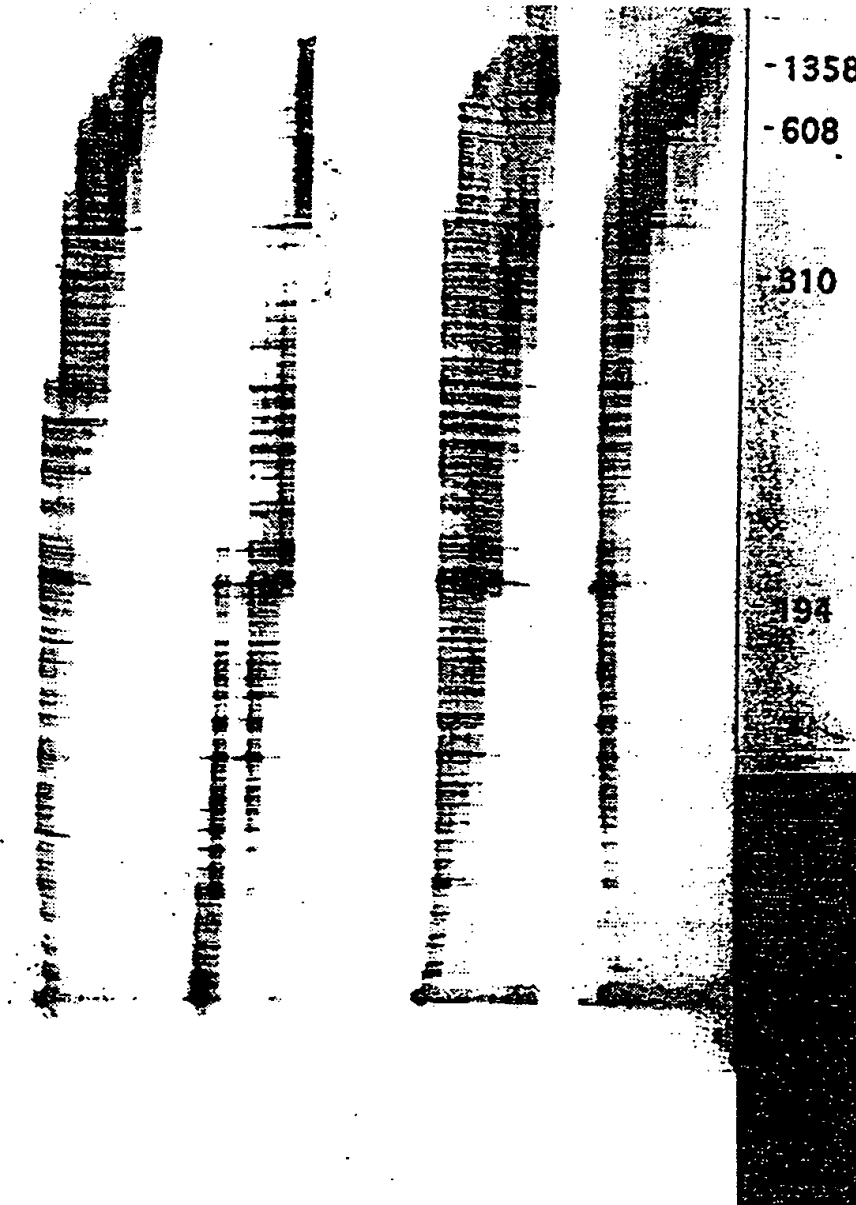


FIG. 5



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FIG. 6

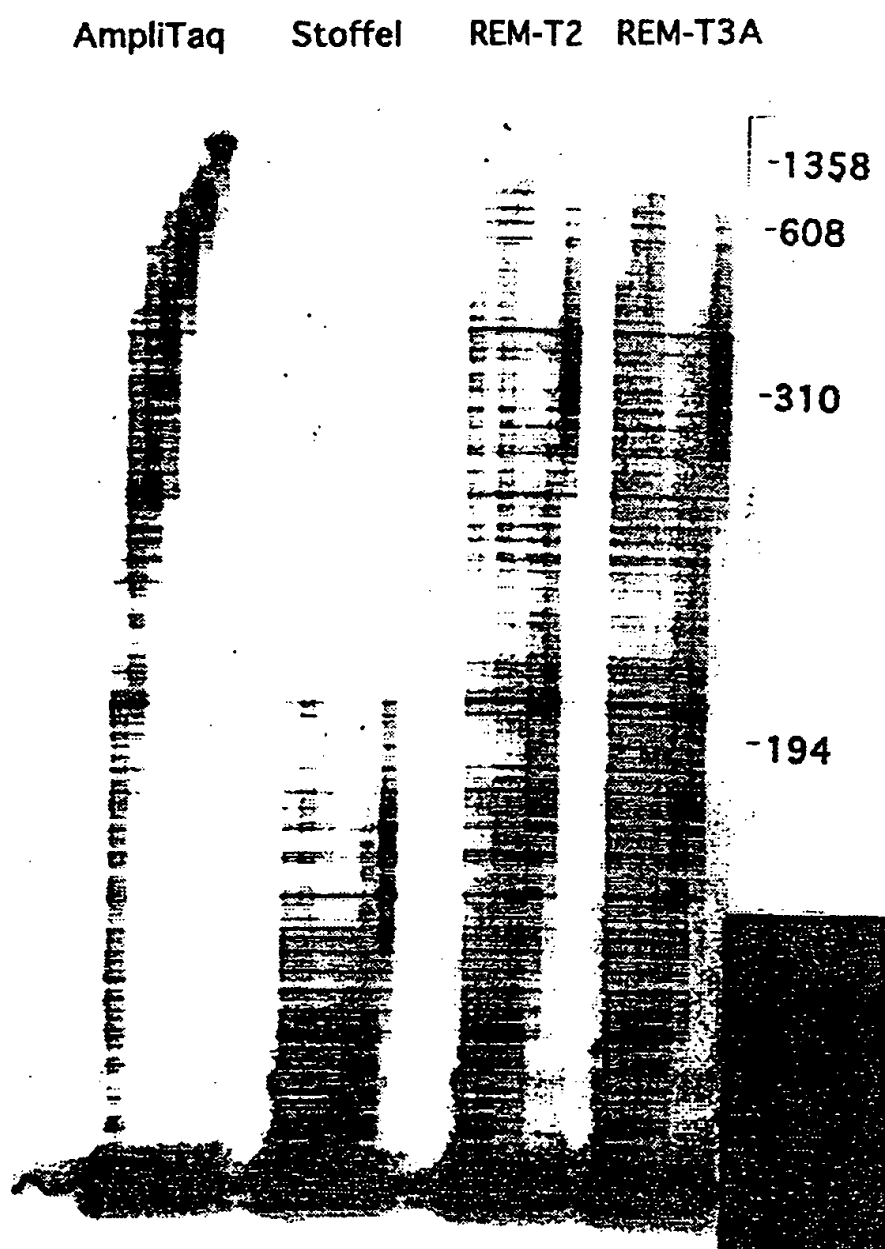
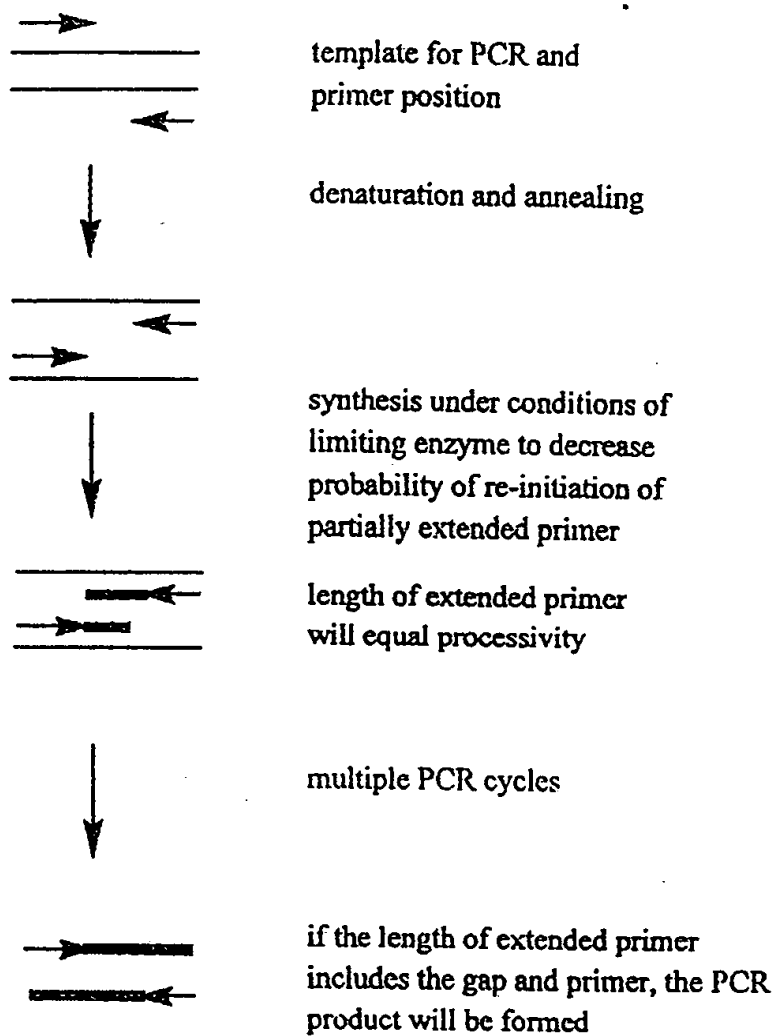


FIG. 7**PCR Analysis of Processivity**

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FIG. 8

